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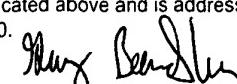
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APPLICATION  
FOR  
UNITED STATES LETTERS PATENT

APPLICANT: GARY RUVKUN AND KAVEH ASHRAFI

TITLE : POLYNUCLEOTIDE AND POLYPEPTIDE FAT  
METABOLISM REGULATORS AND USES  
THEREOF

POLYNUCLEOTIDE AND POLYPEPTIDE FAT METABOLISM

5                   REGULATORS AND USES THEREOF

**Cross-reference to related application**

This application claims the benefit of U.S. provisional application number  
60/395,159, filed on July 11, 2002.

10

**Background of the Invention**

In general, this invention relates to nucleic acid and amino acid sequences involved in fat metabolism regulation and the use of these sequences as targets for the diagnosis, treatment, and prevention of obesity and obesity-related diseases. In addition, the invention relates to screening  
15 methods for identifying modulators of body fat metabolism and the development of treatments for obesity and obesity-related diseases.

Fat metabolism is controlled by a regulatory loop that exists between the central nervous system (CNS) and adipocytes. Adipocytes are specialized cells that store energy in the form of fat droplets, composed primarily of triglycerides. These fat droplets are thought to form by pinching off from membranes of the endoplasmic reticulum. Access to these fat stores is regulated by a protective protein coat, which limits their exposure to cellular lipases, enzymes that breakdown fat. Adipocytes communicate with the CNS via peptide and hormonal signals that carry information regarding the peripheral energy state. In response to these signals, the CNS controls food seeking or satiety behaviors in order to maintain energy homeostasis.

20                  Large gaps remain in our understanding of the cell biology of fat storage, fat droplet biogenesis, and fat droplet size regulation. Moreover, the global regulators of fat metabolism, the interplay of food signals and hormones, and the genetic and environmental factors that influence body weight are still poorly understood. Addressing these deficits is crucial given the devastating impact of obesity on human health throughout the developed world. The  
25                  dysregulation of body weight is associated with obesity, atherosclerosis, type II diabetes mellitus, and osteoarthritis of body joints. Conservative estimates of economic costs associated with the adverse health effects of obesity range between 2% to 7% of total health costs in the

developed world. In the United States, for example, diabetes, one of the diseases associated and exacerbated by obesity, is thought to affect over 16 million individuals at an annual cost of over 92 billion dollars.

As current therapies offer limited effectiveness in treating obesity and obesity-related  
5 disease, a need exists for new therapeutic targets.

### **Summary of the Invention**

As described below, the present invention features nucleic acids and polypeptides that regulate fat metabolism.

10 In a first aspect, the invention generally features a method for identifying a nucleic acid molecule encoding a fat metabolism regulator polypeptide whose inactivation results in an alteration in nematode fat content or localization. The method includes the steps of: (a) providing a mutagenized nematode; (b) contacting the nematode with a dye that stains body fat (e.g., Nile Red); and (c) comparing the body fat staining of the mutagenized nematode to a  
15 control nematode, where a mutation in a nucleic acid molecule encoding a fat metabolism regulator polypeptide is identified by an alteration in nematode fat content or localization. In one embodiment of this method, the mutagenized nematode includes a mutation, prior to mutagenesis, in a fat metabolism regulator nucleic acid molecule.

In a second aspect, the invention generally features a method for identifying a nucleic  
20 acid molecule that encodes a fat metabolism regulator polypeptide whose inactivation results in an alteration in nematode fat content or localization. The method includes the steps of: (a) contacting a nematode with a candidate inhibitory nucleic acid molecule (e.g., dsRNA, siRNA, or antisense); (b) contacting the nematode with a dye that stains body fat (e.g., Nile Red); and (c) comparing the body fat staining of the nematode contacted with the inhibitory nucleic acid  
25 molecule (e.g., dsRNA, siRNA, or antisense) to a control nematode, where an alteration in body fat staining identifies the sense nucleic acid molecule corresponding to an antisense strand of the inhibitory nucleic acid molecule (e.g., dsRNA, siRNA, or antisense), as a nucleic acid molecule encoding a fat metabolism regulator polypeptide whose inactivation results in an alteration in nematode fat content or localization. In one embodiment, the nematode includes a mutation in

*rrf-3*. In another embodiment, the nematode includes a mutation in a fat metabolism regulator nucleic acid molecule (e.g., *lpo-1*, *lpo-2*, *lpo-3*, *lpo-4*, *lpo-5*, *lpo-6*, and *lpo-7*).

In a third aspect, the invention generally features a method for identifying a candidate compound that modulates fat metabolism. The method includes the steps of: (a) providing a cell (e.g., a nematode cell or a mammalian cell) expressing a fat metabolism regulator nucleic acid molecule selected from the group consisting of those encoding a polypeptide listed in Tables V, VI, VII, XII, XIII, or XIV; (b) contacting the cell with a candidate compound; and (c) comparing the expression of the nucleic acid molecule in the cell contacted with the candidate compound with the expression of the nucleic acid molecule in a control cell, where an alteration in the expression identifies the candidate compound as a candidate compound that modulates fat metabolism.

In a fourth aspect, the invention generally features a method for identifying a candidate compound that modulates fat metabolism. The method includes the steps of: (a) providing a nematode cell expressing a fat metabolism regulator nucleic acid molecule; (b) contacting the nematode cell with a candidate compound; and (c) comparing the expression of the nucleic acid molecule in the cell contacted with the candidate compound with the expression of the nucleic acid molecule in a control cell, where an alteration in the expression identifies the candidate compound as a candidate compound that modulates fat metabolism.

In one embodiment of the third or fourth aspects, the screening method identifies a compound that increases or decreases the transcription of the fat metabolism regulator nucleic acid molecule. In another embodiment, the screening method identifies a compound that increases or decreases translation of an mRNA transcribed from the fat metabolism regulator nucleic acid molecule. In yet another embodiment, the compound is a member of a chemical library. In yet another embodiment, the nematode cell is in a nematode. In yet other embodiments, one or more of the fat metabolism regulator nucleic acids are selected from the group consisting of those listed in Tables V, VI, VII, XII, XIII, and XIV.

In a fifth aspect, the invention generally features a method for identifying a candidate compound that modulates fat metabolism. The method includes the steps of: (a) providing a cell (e.g., a nematode cell or a mammalian cell) expressing a fat metabolism regulator polypeptide selected from the group consisting of one or more of those listed in Table V, VI, VII, XII, XIII,

and XIV; (b) contacting the cell with a candidate compound; and (c) comparing the biological activity of the fat metabolism regulator polypeptide in the cell contacted with the candidate compound to a control cell, where an alteration in the biological activity of the fat metabolism regulator polypeptide identifies the candidate compound as a candidate compound that modulates fat metabolism.

In a sixth aspect, the invention generally features a method for identifying a candidate compound that modulates fat metabolism. The method includes the steps of: (a) providing a nematode cell expressing a fat metabolism regulator polypeptide; (b) contacting the nematode cell with a candidate compound; and (c) comparing the biological activity of the fat metabolism regulator polypeptide in the nematode cell contacted with the candidate compound to a control cell, where an alteration in the biological activity of the fat metabolism regulator polypeptide identifies the candidate compound as a candidate compound that modulates fat metabolism.

In one embodiment, the fat metabolism regulator polypeptide is an endogenous regulator polypeptide. In another embodiment, the fat metabolism regulator polypeptide is a polypeptide selected from the group consisting of one or more of those listed in Tables XII, XIII, and XIV. In yet another embodiment, biological activity is monitored with an enzymatic assay, an immunological assay, or by detecting fat levels. In yet another embodiment, the nematode cell is in a nematode.

In a seventh aspect, the invention generally features a method for identifying a candidate compound that modulates fat metabolism. The method includes the steps of: (a) contacting a nematode with a candidate compound and a dye that stains body fat; and (b) comparing staining by the dye in the nematode contacted with a candidate compound to a control nematode, where an alteration in the staining identifies the candidate compound as a candidate compound that modulates fat metabolism. In one embodiment, the nematode includes a mutation in a fat metabolism regulator nucleic acid molecule (e.g., *lpo-1*, *lpo-2*, *lpo-3*, *lpo-4*, *lpo-5*, *lpo-6*, and *lpo-7*).

In an eighth aspect, the invention generally features a microarray consisting of at least two fat metabolism regulator nucleic acids or fragments thereof, where inactivation of each of the fat metabolism regulator nucleic acids results in a decrease in fat content of an organism (e.g., *C. elegans*, a mammal, or a human) compared to a control organism. In one embodiment,

at least one of the fat metabolism regulator nucleic acids is chosen from the group consisting of one or more of the nucleic acids that encode polypeptides listed in Tables V, VI, IX, X, XII, and XIII. In another embodiment, the inactivation does not reduce the viability of an organism.

In a ninth aspect, the invention generally features a microarray consisting of at least two fat metabolism regulator nucleic acids or fragments thereof, where inactivation of each of the fat metabolism regulator nucleic acids results in an increase in fat content of an organism (e.g., *C. elegans*, a mammal, or a human) compared to a control organism. In one embodiment, at least one of the fat metabolism regulator nucleic acids is chosen from the group consisting of one or more of the nucleic acids that encode polypeptides listed in Tables VII, XI, and XIV.

In a tenth aspect, the invention generally features a microarray consisting of at least two of the fat metabolism regulator polypeptide molecules or fragments thereof, where inactivation of each of the fat metabolism regulator polypeptides results in a decrease in fat content of an organism compared to a control organism (e.g., *C. elegans*, a mammal, or a human). In one embodiment, the polypeptides are chosen from the group consisting of one or more of those listed in Tables V, VI, IX, X, XII, and XIII.

In an eleventh aspect, the invention generally features a microarray consisting of at least two of the fat metabolism regulator polypeptides of an organism or fragments thereof, where inactivation of the fat metabolism regulator polypeptides results in an increase in fat content of an organism (e.g., *C. elegans*, a mammal, or a human) compared to a control organism. In one embodiment, at least one of the polypeptides is chosen from the group consisting of one or more of the polypeptides listed in Tables VII, XI, and XIV.

In a twelfth aspect, the invention generally features a method of identifying a candidate compound that modulates fat metabolism. The method includes (a) contacting a cell with a candidate compound; (b) obtaining mRNA from said cell; (c) contacting a microarray of the invention with a candidate compound; and (d) detecting an alteration in cellular mRNA levels of a fat metabolism regulator nucleic acid molecule in said cell contacted with said candidate compound compared to a control cell; where the alteration identifies the candidate compound as a candidate compound that modulates fat metabolism.

In a thirteenth aspect, the invention generally features a method of identifying a candidate compound that modulates fat metabolism. The method includes (a) contacting the microarray of

the invention with a candidate compound; and (b) detecting binding of the candidate compound to a fat metabolism regulator polypeptide; where the binding identifies the compound as a candidate compound that modulates fat metabolism.

In a fourteenth aspect, the invention generally features a purified nucleic acid library, where at least 3%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 95-99% of the total nucleic acids in the library encode fat metabolism regulator polypeptides. In one embodiment, the nucleic acids in the library are carried in a vector. In another embodiment, each of the nucleic acids in the library is fused to a reporter gene. In yet another embodiment, the library includes at least one of the fat metabolism regulator nucleic acids selected from the nucleic acids that encode the polypeptides consisting of one or more of those listed in Tables XII, XIII, or XIV.

In a fifteenth aspect, the invention generally features a method of identifying a candidate compound that modulates fat metabolism. The method includes the steps of: a) contacting a cell including one member of the library described above; and b) measuring the expression of the reporter gene; and c) comparing the level of reporter gene expression in the cell contacted with the candidate compound with a control cell not contacted with the candidate compound, where an alteration in the level of the reporter gene expression identifies the candidate compound as a compound that modulates fat metabolism.

In a sixteenth aspect, the invention generally features an isolated polypeptide including an amino acid sequence having at least 40%, 50%, 60%, 70%, 80%, 90%, or even 95-99% identity to the amino acid sequence of a polypeptide selected from the group consisting of one or more of those listed in Tables XV, XVI, and XVII, where expression of the polypeptide in an organism affects the regulation of fat metabolism in the organism. In one embodiment, the isolated polypeptide of this aspect includes the amino acid sequence of a polypeptide selected from the group consisting of those listed in Tables XV, XVI, and XVII.

In a seventeenth aspect, the invention generally features an isolated nucleic acid molecule including a nucleotide sequence having at least 40%, 50%, 60%, 70%, 80%, 90%, or even 95-99% identity to the nucleotide sequence of a nucleic acid molecule selected from the group consisting of one or more of those that encode the polypeptides listed in Tables XV, XVI, and XVII, where expression of the nucleic acid molecule in an organism affects the regulation of fat

metabolism in the organism. In one embodiment of this aspect, the nucleic acid molecule includes the nucleotide sequence of a nucleic acid molecule selected from the group consisting of those that encode the polypeptides listed in Tables XV, XVI, and XVII or a complement thereof, or a fragment having the biological activity thereof. In addition, the invention includes a vector or a host cell including the isolated nucleic acid molecule of this aspect.

5 In an eighteenth aspect, the invention generally features a transgenic animal (e.g., a *C. elegans*, mammal, or rodent) expressing a fat metabolism regulator nucleic acid sequence, the nucleic acid sequence being selected from the group consisting of those that encode the polypeptides listed in Tables XV, XVI, and XVII.

10 In a nineteenth aspect, the invention generally features an organism (e.g., a *C. elegans*, mammal, or rodent) including a mutation in a fat metabolism regulator nucleic acid sequence the nucleic acid sequence being selected from the group consisting of those that encode the polypeptides listed in Tables XV, XVI, and XVII.

15 In a twentieth aspect, the invention generally features a double-stranded RNA (e.g., siRNA) corresponding to at least a portion of a fat metabolism regulator nucleic acid molecule of an organism the nucleic acid molecule being selected from the group consisting of those that encode the polypeptides listed in Tables XV, XVI, and XVII, where the double-stranded RNA is capable of decreasing the level of protein encoded by the fat metabolism regulator nucleic acid molecule.

20 In a twenty-first aspect, the invention generally features an antisense nucleic acid molecule, where the nucleic acid molecule is complementary to at least six nucleotides of a nucleic acid molecule selected from the group consisting of those that encode the polypeptides listed in Tables XV, XVI, and XVII, and where the antisense nucleic acid is capable of decreasing expression from the nucleic acid molecule to which it is complementary.

25 In a twenty-second aspect, the invention generally features an isolated polypeptide including an amino acid sequence having at least 35%, 40%, 50%, 60%, 70%, 80%, 90%, or even 95-99% identity to the amino acid sequence of LPO-1 (SEQ ID NO.:2), where expression of the polypeptide in an organism (e.g., a *C. elegans*, mammal, rodent, or human) affects the regulation of fat metabolism in the organism. In one embodiment, the polypeptide includes the 30 amino acid sequence of LPO-1 (SEQ ID NO:2).

In a twenty-third aspect, the invention generally features an isolated nucleic acid molecule having at least 35%, 40%, 50%, 60%, 70%, 80%, 90%, or even 95-99% identity to the nucleotide sequence of *lpo-1* (SEQ ID NO:1), where expression of the nucleic acid molecule in an organism (e.g., a *C. elegans*, mammal, or human) affects the regulation of fat metabolism in 5 the organism. In one embodiment, the nucleic acid molecule includes the nucleotide sequence of *lpo-1* (SEQ ID NO:1) or a complement thereof. In another embodiment the invention features a vector or host cell including the isolated nucleic acid molecule of the twenty-third aspect.

In a twenty-fourth aspect, the invention generally features an antibody that specifically binds to the LPO-1 (SEQ ID NO:2) polypeptide.

10 In a twenty-fifth aspect, the invention generally features an isolated polypeptide including an amino acid sequence having at least 45%, 50%, 60%, 70%, 80%, 90%, or even 95-99% identity to the amino acid sequence of LPO-3 (SEQ ID NO:4), where expression of the polypeptide in an organism (e.g., a *C. elegans*, mammal, or human) affects the regulation of fat metabolism in the organism.

15 In a twenty-sixth aspect, the invention generally features an isolated nucleic acid molecule having at least 45%, 50%, 60%, 70%, 80%, 90%, or even 95-99% % identity to the nucleotide sequence of *lpo-3* (SEQ ID NO:3), where expression of the nucleic acid molecule in an organism affects the regulation of fat metabolism in the organism.. In one embodiment, , the nucleic acid molecule includes the nucleotide sequence of *lpo-3* or a complement thereof. In 20 another embodiment of this aspect, the nucleic acids are included in a vector or a host cell.

In a twenty-seventh aspect, the invention generally features a method for diagnosing an organism (e.g., a human) having, or having a propensity to develop, a disease associated with fat metabolism regulation, obesity, or obesity-related diseases. The method includes detecting an alteration in the sequence of a fat metabolism regulator nucleic acid molecule relative to a wild-type sequence of said fat metabolism regulator nucleic acid molecule, the molecule being selected from the group consisting of one or more of those that encode the polypeptides listed in 25 Tables XII, XIII, and XIV.

30 In a twenty-eighth aspect, the invention generally features a method for diagnosing an organism (e.g., a human) having, or having a propensity to develop, a disease associated with fat metabolism regulation, obesity, or an obesity-related disease. The method includes detecting an

alteration in the expression of a fat metabolism regulator nucleic acid molecule or polypeptide relative to the wild type level of expression of said fat metabolism regulator nucleic acid molecule or polypeptide, the nucleic acid or polypeptide being selected from the group consisting of those listed in Tables XII, XIII, and XIV.

5        In a twenty-ninth aspect, the invention generally features a method for diagnosing an organism (e.g., a human) having, or having a propensity to develop, a disease associated with fat metabolism regulation, obesity, or an obesity-related disease. The method includes detecting an alteration in the biological activity of a fat metabolism regulator polypeptide relative to the wild-type level of activity.

10      In a thirtieth aspect, the invention generally features a collection of primer sets, each of the primer sets including at least two primers that bind to a fat metabolism regulator nucleic acid molecule that encodes a polypeptide selected from the group consisting of those listed in Tables IX, X, XI, XII, XIII, and IV under high stringency conditions, the collection including at least two primer sets. In one embodiment, the binding detects an alteration in a fat metabolism 15     regulator nucleic acid molecule. In another embodiment, the primer sets can be used to amplify a fat metabolism regulator nucleic acid molecule.

In a thirty-first aspect, the invention generally features a method for ameliorating or delaying a fat metabolism or obesity disorder in an organism (e.g., a human). The method includes contacting the organism with an inhibitory nucleic acid molecule (e.g., dsRNA, siRNA, 20    or antisense) whose antisense strand complements a portion of a fat metabolism regulator nucleic acid molecule selected from the group consisting of those that encode the polypeptides listed in Tables XII, XIII, and IV.

In a thirty-second aspect, the invention generally features a method for ameliorating or delaying a fat metabolism or obesity disorder in an organism (e.g., a human). The method 25    includes contacting the organism with a fat metabolism regulator nucleic acid molecule selected from the group consisting of those that encode the polypeptides listed in Tables XII, XIII, and IV.

In a thirty-third aspect, the invention generally features a pharmaceutical composition including fat metabolism regulator polypeptides or portions thereof, selected from the group

consisting of those that encode the polypeptides listed in Tables XII, XIII, and IV, that treat a fat metabolism or obesity disorder.

In a thirty-fourth aspect, the invention generally features a pharmaceutical composition including a fat metabolism regulator nucleic acid molecule or portion thereof, selected from the 5 group consisting of those that encode the polypeptides listed in Tables XII, XIII, and IV, that treats a fat metabolism or obesity disorder.

In preferred embodiments of any of the above aspects, any one or more of the nucleic acids or polypeptides selected from the group consisting of those listed in Tables V, VI, VII, IX, X, XI, XII, XIII, XIV, XV, XVI, XVII, XVIII, XIX, and XX may be used.

10 By “isolated polynucleotide” is meant a nucleic acid (e.g., a DNA) that is free of the genes which, in the naturally-occurring genome of the organism from which the nucleic acid molecule of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that exists as a separate  
15 molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. In addition, the term includes an RNA molecule which is transcribed from a DNA molecule, as well as a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

By “polypeptide” is meant any chain of amino acids, regardless of length or post-  
20 translational modification (for example, glycosylation or phosphorylation).

By an “isolated polypeptide” is meant a polypeptide of the invention that has been separated from components that naturally accompany it. Typically, the polypeptide is isolated when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more  
25 preferably at least 90%, and most preferably at least 99%, by weight, a polypeptide of the invention. An isolated polypeptide of the invention may be obtained, for example, by extraction from a natural source, by expression of a recombinant nucleic acid encoding such a polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, for example, column chromatography, polyacrylamide gel electrophoresis, or by HPLC analysis.

By "substantially identical" is meant a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). Preferably, such a sequence is at least 60%, more preferably 80%, 5 and most preferably 90% or even 95% identical at the amino acid level or nucleic acid to the sequence used for comparison.

Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705, BLAST, BESTFIT, GAP, 10 or PILEUP/Prettybox programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to 15 determining the degree of identity, a BLAST program may be used, with a probability score between  $e^{-3}$  and  $e^{-100}$  indicating a closely related sequence.

By "transformed cell" is meant a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a polynucleotide molecule encoding (as used herein) a polypeptide of the invention.

20 By "positioned for expression" is meant that the polynucleotide of the invention (e.g., a DNA molecule) is positioned adjacent to a DNA sequence which directs transcription and translation of the sequence (i.e., facilitates the production of, for example, a recombinant polypeptide of the invention, or an RNA molecule).

By "purified antibody" is meant an antibody which is at least 60%, by weight, free from 25 proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably 90%, and most preferably at least 99%, by weight, antibody. A purified antibody of the invention may be obtained, for example, by affinity chromatography using a recombinantly-produced polypeptide of the invention and standard techniques.

By “specifically binds” is meant a compound or antibody which recognizes and binds a polypeptide of the invention but which does not substantially recognize and bind other molecules in a sample, for example, a biological sample, which naturally includes a polypeptide of the invention.

5 By “derived from” is meant isolated from or having the sequence of a naturally-occurring sequence (e.g., a cDNA, genomic DNA, synthetic, or combination thereof).

By “immunological assay” is meant an assay that relies on an immunological reaction, for example, antibody binding to an antigen. Examples of immunological assays include ELISAs, Western blots, immunoprecipitations, and other assays known to the skilled artisan.

10 By “inhibitory nucleic acid” is meant a nucleic acid that reduces or eliminates expression or biological activity of a gene or protein of interest. “Inhibitory nucleic acids” include, without limitation, antisense nucleic acids, double stranded RNAs (dsRNA), or small interfering RNAs (siRNA), or analogs thereof.

15 By “anti-sense” is meant a nucleic acid, or analog thereof, regardless of length, that is complementary to the coding strand or mRNA of a nucleic acid sequence. In one embodiment, an antisense RNA is introduced to an individual cell, tissue, organ, or to a whole animals. Desirably the anti-sense nucleic acid is capable of decreasing the expression or biological activity of a nucleic acid or amino acid sequence. In one embodiment, the decrease in expression or biological activity is at least 10%, relative to a control, more desirably 25%, and most desirably 50%, 60%, 70%, 80%, 90%, or more. The anti-sense nucleic acid may contain a modified backbone, for example, phosphorothioate, phosphorodithioate, or other modified backbones known in the art, or may contain non-natural internucleoside linkages.

20 By “double stranded RNA” is meant a complementary pair of sense and antisense RNAs regardless of length. In one embodiment, these dsRNAs are introduced to an individual cell, tissue, organ, or to a whole animals. For example, they may be introduced systemically via the bloodstream. Desirably, the double stranded RNA is capable of decreasing the expression or biological activity of a nucleic acid or amino acid sequence. In one embodiment, the decrease in expression or biological activity is at least 10%, relative to a control, more desirably 25%, and most desirably 50%, 60%, 70%, 80%, 90%, or more. The anti-sense nucleic acid may contain a

modified backbone, for example, phosphorothioate, phosphorodithioate, or other modified backbones known in the art, or may contain non-natural internucleoside linkages.

By “siRNA” is meant a double stranded RNA that complements a region of an mRNA. Optimally, an siRNA is 22-24 nucleotides in length and has a 2 base overhang at its 3’ end.

- 5 These dsRNAs can be introduced to an individual cell or to a whole animal, for example, they may be introduced systemically via the bloodstream. Such siRNAs are used to downregulate mRNA levels or promoter activity. In one embodiment, the decrease in expression or biological activity is at least 10%, relative to a control, more desirably 25%, and most desirably 50%, 60%, 70%, 80%, 90%, or more. The siRNA may contain a modified backbone, for example,
- 10 phosphorothioate, phosphorodithioate, or other modified backbones known in the art, or may contain non-natural internucleoside linkages.

By “hybridize” is meant pair to form a double-stranded molecule between complementary polynucleotide sequences (e.g., genes listed in Tables 1-4 and 7), or portions thereof, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987)

- 15 *Methods Enzymol.* 152:399; Kimmel, A. R. (1987) *Methods Enzymol.* 152:507) For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high
- 20 stringency hybridization can be obtained in the presence of at least about 35% formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at least about 42°C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of
- 25 carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30°C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37°C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 µg/ml denatured salmon sperm DNA
- 30 (ssDNA). In a most preferred embodiment, hybridization will occur at 42°C in 250 mM NaCl,

25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature.

- 5 As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25°C, more preferably of at least about 42°C,
- 10 and most preferably of at least about 68°C. In a preferred embodiment, wash steps will occur at 25°C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a most preferred embodiment, wash steps will occur at 68°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to
- 15 those skilled in the art. Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (*Science* 196:180, 1977); Grunstein and Hogness (*Proc. Natl. Acad. Sci., USA* 72:3961, 1975); Ausubel et al. (*Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001); Berger and Kimmel (*Guide to Molecular Cloning Techniques*, 1987, Academic Press, New York); and Sambrook et al.,
- 20 *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York.

By “binds” is meant a compound or antibody which recognizes and binds a polypeptide of the invention but which does not substantially recognize and bind other different molecules in a sample, for example, a biological sample, which naturally includes a polypeptide of the invention.

- 25 By “ortholog” is meant a polypeptide or nucleic acid molecule of an organism that is highly related to a reference protein, or nucleic acid sequence, from another organism. An ortholog is functionally related to the reference protein or nucleic acid sequence. In other words, the ortholog and its reference molecule would be expected to fulfill similar, if not equivalent, functional roles in their respective organisms. For example, a *C. elegans* lipase and its
- 30 mammalian ortholog would both be expected to fulfill the enzymatic function of lipases in their

respective organisms. It is not required that an ortholog, when aligned with a reference sequence, have a particular degree of amino acid sequence identity to the reference sequence. A protein ortholog might share significant amino acid sequence identity over the entire length of the protein, for example, or, alternatively, might share significant amino acid sequence identity 5 over only a single functionally important domain of the protein. Orthologs may be identified using methods provided herein. The functional role of an ortholog may be assayed using methods well known to the skilled artisan, and described herein. For example, function might be assayed *in vivo* or *in vitro* using a biochemical, immunological, or enzymatic assays; transformation rescue, Nile Red or BODIPY assays for the effect of gene inactivation on fat 10 content, storage, or mobilization; such fat content assays, as described herein, may be carried out in a whole animal (e.g., *C. elegans*) or in tissue culture; function may also be assayed by gene inactivation (e.g., by RNAi, siRNA, or gene knockout), or gene over-expression, as well as by other methods.

By “fat metabolism” is meant, for example, fat storage, fat deposition, fat breakdown, fat 15 droplet biogenesis, fat mobilization, or the increase, decrease, or maintenance of the fat content of an organism.

By “fat metabolism regulator polypeptide” is meant a polypeptide that modulates fat metabolism, for example, fat storage, fat deposition, fat breakdown, fat droplet biogenesis, fat mobilization, or the fat content of an organism. A fat metabolism regulator polypeptide has at 20 least 50%, 60%, 70% amino acid sequence identity to the proteins encoded by the nucleic acid sequences listed in, for example, Tables V, VI, VII, IX, X, XI, XII, XIII, XIV, XV, XVI, and XVII. More desirably, a fat metabolism regulator polypeptide would have at least 75%, 80%, 85% amino acid sequence identity to the proteins encoded by the nucleic acid sequences listed in, for example, Tables V, VI, VII, IX, X, XI, XII, XIII, XIV, XV, XVI, and XVII. A fat 25 metabolism regulator polypeptide could have at least 90%, 95%, or even 97% identity with polypeptide encoded by a nucleic acid sequence listed in, for example, Tables V, VI, VII, IX, X, XI, XII, XIII, XIV, XV, XVI, and XVII.

By “fat metabolism regulator nucleic acid” is meant a nucleic acid that encodes a fat metabolism regulator polypeptide. Such polypeptides are encoded by the nucleic acid sequences 30 listed in, for example, Tables V, VI, VII, IX, X, XI, XII, XIII, XIV, XV, XVI, and XVII.

By “transgene” is meant any piece of DNA which is inserted by artifice into a cell and typically becomes part of the genome of the organism which develops from that cell. Such a transgene may include a gene which is partly or entirely heterologous (i.e., foreign) to the transgenic organism, or may represent a gene homologous to an endogenous gene of the

5 organism.

By “transgenic” is meant any cell which includes a DNA sequence which is inserted by artifice into a cell and becomes part of the genome of the organism which develops from that cell. As used herein, the transgenic organisms are generally transgenic vertebrates, such as, zebrafish, mice, and rats, and the DNA (transgene) is inserted by artifice into the nuclear

10 genome.

“Cell” as used herein may be a single-cellular organism, cell from a multi-cellular organism, or it may be a cell contained in a multi-cellular organism.

“Differentially expressed” means a difference in the expression level of a nucleic acid. This difference may be either an increase or a decrease in expression, when compared to control

15 conditions.

“Microarray” means a collection of nucleic acids or polypeptides from one or more organisms arranged on a solid support (for example, a chip, plate, or bead). These nucleic acids or polypeptides may be arranged in a grid where the location of each nucleic acid or polypeptide remains fixed to aid in identification of the individual nucleic acids or polypeptides. A

20 microarray may include, for example, nucleic acids representing all, or a subset, of the open reading frames of an organism, or of the polypeptides that those open reading frames encode. In one embodiment, the nucleic acids of the array are defined as having a common region of the genome having limited homology to other regions of an organism’s genome. A microarray may also be enriched for a particular type of gene. In one example, a “microarray of fat metabolism

25 regulator nucleic acids or polypeptides” may be enriched for fat metabolism regulator nucleic acids or polypeptides so that, for example, it comprises at least 5%, 10%, 15%, 20%, 22%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97% or even 99% fat metabolism regulator genes or their encoded fat metabolism regulator polypeptides. In one example, a “microarray of fat metabolism regulator nucleic acids or polypeptides” comprises the *C. elegans* nucleic acids listed

30 in Tables V, VI, VII, IX, X, XI, XII, XIII, XIV, XV, XVI, and XVII; or the mammalian nucleic

acids listed in Table IX, X, XI, XII, XIII, XIV, XV, XVI, or XVII, or the polypeptides they encode.

“Primer set” means a set of oligonucleotides that may be used, for example, for PCR. A primer set would consist of at least 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 30, 40, 50, 60, 80, 100, 200, 250, 300, 400, 500, 600, or more primers.

“Therapeutic compound” means a substance that has the potential of affecting the function of an organism. Such a compound may be, for example, a naturally occurring, semi-synthetic, or synthetic agent. For example, the test compound may be a drug that targets a specific function of an organism. A test compound may also be an antibiotic or a nutrient. A therapeutic compound may decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of disease, disorder, or infection in a eukaryotic host organism.

The invention provides a number of targets that are useful for the development of drugs to treat obesity and the dysregulation of fat metabolism. In addition, the methods of the invention provide a facile means to identify therapies that are safe for use in eukaryotic host organisms (i.e., compounds which do not adversely affect the normal development, physiology, or fertility of the organism). In addition, the methods of the invention provide a route for analyzing virtually any number of compounds for effects on fat metabolism with high-volume throughput, high sensitivity, and low complexity. The methods are also relatively inexpensive to perform and enable the analysis of small quantities of active substances found in either purified or crude extract form.

Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

#### Brief Description of the Drawings

Figure 1A shows the chemical structure of Nile Red.

Figure 1B is a Nomarski photomicrograph showing a wild-type nematode grown on Nile Red, and a photomicrograph showing Nile Red staining in a wild-type nematode grown on Nile Red.

Figure 2A shows the chemical structure of C1 and C5 BODIPY fluorophore (Molecular Probes, D-3823 and D-3825) labeled fatty acids.

Figure 2B is a Nomarski photomicrograph showing a wild-type nematode grown on C1 BODIPY fat.

Figure 2C is a photomicrograph showing GFP fluorescence in a wild-type nematode grown on C1 BODIPY fat.

5 Figure 2D is a photomicrograph showing Sudan black staining in a wild-type nematode.

Figure 3A is a photomicrograph showing a nematode co-stained with Nile Red and BODIPY-labeled fatty acids. In this image a rhodamine overlay (visualizing Nile Red) is superimposed on a Nomarski image (400x mag). The anterior of the animal is at the upper right hand corner of the panel.

10 Figure 3B is a photomicrograph showing C<sub>1</sub>-BODIPY 500/510 C<sub>12</sub> fatty acid staining of the same nematode pictured in Figure 3A. In this image a GFP overlay is superimposed on a Nomarski image. An identical staining pattern was observed when C<sub>8</sub>-BODIPY 500/510 C<sub>5</sub> was used.

Figures 4A-4C show nematodes photographed under equal exposure conditions.

15 Figure 4A is a photomicrograph showing Nile Red staining in a wild-type nematode. Figure 4B is a photomicrograph showing Nile Red staining in a *tph-1(mg280)II* (Sze et al., *Nature* 403:560-4, 2000) (serotonin-deficient) nematode. Figure 4C is a photomicrograph showing Nile Red staining in a *tub-1(nr2004)* nematode.

20 Figure 5A is a photomicrograph showing Nile Red fat staining in non-starved young adult wild-type *C. elegans* (160X mag).

Figure 5B is a photomicrograph showing Nile Red fat staining in a non-starved young adult insulin receptor *daf-2(e1370)* mutant nematode (160X mag)..

Figure 5C is a photomicrograph showing Nile Red fat staining in a *daf-16(mgDf47);daf-2(e1370)* mutant nematode (160X mag).

25 Figure 5D is a photomicrograph showing Nile Red fat staining in a *daf-2(e1370);daf-3(mgDf90)* mutant nematode (160X mag).

Figure 5E is a bar graph showing quantitation of fat staining in indicated mutant nematodes by measuring pixel intensity and number of Nile Red stained droplets (n=10 nematodes in at least 2 independent experiments. Standard deviation is given as error bars).

Figures 6A-6D are photomicrographs showing that increased fat stores in dauers are detectable by Nile Red staining. Figures 6A and 6C show corresponding rhodamine and Nomarski images, respectively, of a *daf-2(e1370)* animal grown at 25°C to induce dauer formation. Figures 6B and 6D show corresponding rhodamine and Nomarski images,  
5 respectively, of a *daf-2(e1370)* animal grown at 15°C to bypass dauer entry.

Figure 7 is a bar graph showing that differences in Nile Red staining correspond to actual fat content. Total *C. elegans* lipid extracts were separated into triacylglyceride and phospholipid components and their respective constituents were identified and quantitated by gas chromatography. Reported numbers have been normalized to protein content extracted from the  
10 same *C. elegans* (n=2 measurements from two independent extractions; standard deviation is given as error bars).

Figures 8A-8F are photomicrographs showing that mobilization of fat droplets in a nematode can be monitored by Nile Red staining. Figure 8A shows Nile Red staining in a wild-type untreated nematode. Figure 8B shows Nile Red staining in a wild-type nematode treated  
15 with 5-aminoimidazole-4-carbozamide ribonucleoside (AICAR). Figure 8C shows Nile Red staining in a wild-type starved nematode. Figure 8D shows Nile Red staining in an adult *daf-2(e1370)* mutant nematode grown at the permissive temperature of 15° C past the dauer decision stage and then shifted to the non-permissive temperature of 25° C. This nematode has increased  
20 fat content relative to a wild-type nematode. Figure 8E shows Nile Red staining in a *daf-2(e1370)* mutant nematode treated with AICAR. Figure 8F shows Nile Red staining in a starved adult *daf-2(e1370)* mutant nematode grown at 15°C past the dauer decision stage and then shifted to the non-permissive temperature, 25°C. This nematode has increased fat content relative to a wild-type nematode.

Figure 9A is a photomicrograph showing Nile Red staining in a wild-type nematode.

25 Figure 9B is a photomicrograph showing Nile Red staining in an *lpo-1* mutant nematode with increased fat staining.

Figure 9C is a photomicrograph showing Nile Red staining in an *lpo-2* mutant nematode with reduced fat staining.

30 Figure 9D is a photomicrograph showing Nile Red staining in an *lpo-3* mutant nematode with reduced fat staining.

Figure 9E is a photomicrograph showing Nile Red staining in a wild-type nematode (400X magnification).

Figure 9F is a photomicrograph showing Nile Red staining in an *lpo-6* mutant nematode with increased fat droplets (400x magnification).

5 Figure 9G is a photomicrograph showing Nile Red staining in an *lpo-4* mutant nematode with reduced fat staining (400x magnification) and altered Nile Red emission profile. Green emission is observed instead of red.

Figure 10A is a photomicrograph showing C12-BODIPY-labelled fatty acid staining in a wild-type nematode.

10 Figure 10B is a photomicrograph showing Nile Red staining in a wild-type nematode.

Figure 10C is a photomicrograph showing C12-BODIPY-labelled fatty acid staining in a *lpo-1* nematode.

Figure 10D is a photomicrograph showing Nile Red staining in a *lpo-1* nematode.

15 Figure 10E is a photomicrograph showing C12-BODIPY-labelled fatty acid staining in a *lpo-2* nematode.

Figure 10F is a photomicrograph showing Nile Red staining in a *lpo-2* nematode.

Figure 11A is a photomicrograph showing Nile Red staining in a *lpo-1* nematode.

Figure 11B is a photomicrograph showing Nile Red staining in a *lpo-1* nematode transformation rescued by expression of wild-type *lpo-1*.

20 Figure 11C is a schematic diagram showing the structure of the LPO-1 polypeptide.

Figure 11D shows the genomic nucleic acid sequence of *lpo-1* (Genomic Position: chromosome II: 6783394-6787620) (SEQ ID NO:1). The 5,570 nucleic acid sequence includes 545 basepairs upstream of the start codon, ATG (which is highlighted); 4,228 nucleotides of predicted exons (which are shown in upper-case letters) and introns (which are shown in lower case) and 558 basepairs downstream of the stop codon, TGA (which is highlighted).

25 Figure 11E shows the nucleic acid sequence (SEQ ID NO:2) (2592 nucleotides) of the *lpo-1* open reading frame.

Figure 11F shows the predicted amino acid sequence (SEQ ID NO:3) of the LPO-1 protein.

Figure 11G shows an alignment of LPO-1 and the human (accession number: 4507901) (SEQ ID NO:9) and rat (accession number: 6981706) (SEQ ID NO:10) very low density lipoprotein (VLDL) receptors. Identical amino acids are denoted with an asterisk (\*). Conservative substitutions are denoted with a period (.), and substitutions that conserve the charge of the amino acid residues are denoted with a colon (:).

5 Figure 12A is a photomicrograph showing Nile Red staining in an *lpo-3* mutant nematode.

Figure 12B is a photomicrograph showing Nile Red staining in a *lpo-3* nematode transformation rescued by expression of wild-type *lpo-3*.

10 Figure 12C is a schematic diagram showing the structure of the LPO-3 polypeptide.

Figure 12D shows the genomic nucleic acid sequence (SEQ ID NO:4) of *lpo-3* (Genomic Position: chromosome I: 5897000-5903772). The 7,496 nucleotide sequence includes 420 nucleotides upstream of the start codon, ATG (which is highlighted); 6,774 nucleotides of predicted exons (which are shown in capital letters) and introns (which are shown in lower case letters); and 304 nucleotides downstream of the stop codon, TGA (which is highlighted).

15 Figure 12E shows the nucleic acid sequence (SEQ ID NO:5) of the *lpo-3* open reading frame.

Figure 12F shows the amino acid sequence (SEQ ID NO:6) of LPO-3.

20 Figure 12G shows an amino acid sequence alignment of the LPO-3 with the human (Human Multidrug resistance protein 1 (P-glycoprotein-1), accession number: 2506118) (SEQ ID NO:11) and mouse (ATP-binding cassette (P glycoprotein 1), accession number: 6755046) (SEQ ID NO:12) ATP-binding cassette (ABC)-type transporters. The glycine at position 1163, denoted in red, is mutated to aspartic acid in *lpo-3*. Identical amino acids are denoted with an asterisk (\*). Conservative substitutions are denoted with a period (.), and substitutions that conserve the charge of the amino acid residues are denoted with a colon (:).

25 Figures 13A-13F are photomicrographs showing Nile Red staining superimposed on a Nomarski image in wild-type nematodes grown on *E. coli* carrying an RNA interference (RNAi) clone. Figure 13A shows a nematode grown on L4440 vector control RNAi bacteria.

Figure 13B shows reduced straining in a nematode grown on Y49A3A.1 (choline/ethanolamine phosphotransferase) RNAi bacteria. Figure 13C shows reduced

staining in a nematode grown on F08F8.2 (HMG-CoA reductase) RNAi bacteria. Figure 13D shows reduced staining in a nematode grown on Y47D3B.7 (Sterol Response Element Binding Protein (SREBP), a transcription factor required for endogenous sterol synthesis) RNAi bacteria. Figure 13E shows mislocalized staining in a nematode grown on K02D3.2 (steroidogenic acute regulatory related protein (StAr)) RNAi bacteria. Figure 13F shows increased staining in a nematode grown on NHLH2 (neurogenic transcription factor) RNAi bacteria.

### Description of the Invention

The present invention features *C. elegans* fat metabolism regulator genes and polypeptides. Mammalian orthologs of these *C. elegans* genes have also been identified. Because pathways that regulate fat cell biology are likely to be evolutionarily conserved between mammals and nematodes, these mammalian genes provide new targets for the treatment of obesity and obesity-related disease, and the invention also features such methods.

As reported in more detail below, a systematic survey of the *C. elegans* genome using RNAi has identified nematode (and mammalian) genes that regulate fat storage. These fat metabolism regulator genes have been characterized in *C. elegans* and found to define two sets. A first set of fat metabolism regulator genes was defined by those whose inactivation caused a reduced fat (50% or less of wild-type fat level) or altered fat deposition phenotype. A large subset of these fat metabolism regulator genes included those whose inactivation did not result in significant viability, growth, or fertility defects. The mammalian orthologs of these *C. elegans* genes provide attractive therapeutic targets whose inactivation is unlikely to result in adverse side effects. Another attractive subset of therapeutic targets are those *C. elegans* genes and their mammalian orthologs whose inactivation results in a much reduced fat phenotype (20% or less of wild-type fat level) and whose inactivation does not significantly interfere with viability, growth, or fertility defects. Activating mutations in the human orthologs of these genes are likely to underlie human obesity or fat metabolism disorders.

A second useful set of *C. elegans* genes is defined by those whose inactivation results in an increase in nematode fat content. Loss of function or dominant negative mutations in these genes are likely to underlie human obesity or fat metabolism disorders.

A systematic method of identifying fat metabolism regulator genes was used that provides unique advantages over existing methods of gene identification, such as transcriptional profiling studies. In particular, the approach described herein assigns a genetic function in fat metabolism to genes identified in a genome-wide RNAi screen. In contrast, gene array based 5 gene identification implicates a gene in a process by its transcriptional regulation, but fails to assign a function to that gene. The genetic and RNAi approaches described herein test whether a particular gene is required for fat accumulation, thus characterizing the function of the identified gene in *C. elegans* fat metabolism and predicting its role in mammalian fat metabolism. Many of the genes identified (e.g., phosphoenolpyruvate carboxykinase (PEPCK), 3-hydroxyacyl-CoA 10 dehydrogenase, choline/ethanolamine kinase, and sterol response element binding protein, a transcription factor required for endogenous sterol synthesis (SREBP)) are focal points of regulation for their respective multicomponent metabolic pathways (gluconeogenesis,  $\beta$ -oxidation, phospholipid biosynthesis, and sterol metabolism, respectively). The fat phenotypes produced by their inactivation are likely due to significant shifts in metabolism resulting from the 15 perturbation of key regulatory components. Given this identification of known, important components of fat metabolism, it is reasonable to conclude that other metabolic genes identified by this assay also serve as key regulated components of their particular pathways.

### *C. elegans* Strains

20 All strains were maintained as described by Brenner (*Brenner, Genetics* 77:71-94, 1974) at 25°C, except when noted. The *E. coli* used for feeding *C. elegans* was strain OP50. The wild-type reference strain was N2 Bristol. The mutant strains used herein were as follows: *tub-1(nr2004)II* (kindly provided by Carl D. Johnson), *tph-1(mg280)II* (Sze et al., *Nature* 403:560-4, 2000), *pgp-1(pk17)IV*, *pgp-3(pk18)X*, *mrp-1(pk89)X*; *pgp-1(pk17)IV*; *pgp-3(pk18)X*; *mrp-25 1(pk89)* (Broeks et al., *Embo J.* 14:1858-66, 1995).

The following strains were hatched and grown at the permissive temperature 15°C until the L2 stage and then transferred to 25°C: *daf-2(e1370) III*, *daf-2(e1370) III*; *daf-1(m40) IV*, *daf-2(e1370) III*; *daf-3(mgDf90) X*, *daf-2(e1370) III*; *daf-12(sa204) X*, *daf-16(mgDf47) I*; *daf-2(e1370) III*, *daf-2(e1370) III*; *daf-18(mg198) IV*, *daf-16(mgDf47) I*, *pdk-1(sa680) X*, *daf-16(30 mgDf47) I*, *daf-3(mgDf90) X*, *daf-12(m20) X*, *daf-7(e1372) III*, *daf-7(e1372) III*; *daf-12(m20)*

X, *daf-7(e1372)* III; *daf-3(mgDf90)* X, (Paradis et al., *Genes Dev.* 13:1438-52, 1999; Tissenbaum et al., *Genetics* 148:703-17, 1998; Patterson et al., *Genes Dev.* 11:2679-90, 1997; Gottlieb et al., *Genetics* 137:107-20, 1994); many of these strains were provided by the Caenorhabditis Genetic Center.

5

### Detection of Nematode Fat by Nile Red

The vital dye, 9-diethylamino-5H-benzo[ $\alpha$ ]phenoxazine-5-one (Nile Red) (Figure 1A), was used to visualize fat droplets in living nematodes (Figure 1B). Nile Red had previously been used as a vital stain for the detection of intracellular lipid droplets by fluorescence microscopy in cell culture models of fat accumulation (Greenspan et al., *J. Lipid. Res.* 26:781-9, 1985; Greenspan et al., *J. Cell. Biol.* 100:965-73, 1985). Nile Red is non-fluorescent in an aqueous environment, but undergoes a spectral shift in the presence of hydrophobic lipid.

Nile Red was adapted for use in a *C. elegans* *in vivo* genetic screen. Nile Red powder (N-1142 Molecular Probes) was dissolved in acetone at 500  $\mu$ g/ml. It was then diluted in 1X phosphate buffered saline (PBS) and added to nematode growth media (NGM) plates, with lawns of OP50 or RNAi bacteria. The final Nile Red concentration in plates was 0.05  $\mu$ g/ml.

Nematodes were cultured on plates containing *E. coli* OP50 mixed with Nile Red. While no Nile Red fat staining was observed in nematodes maintained on agar plates containing Nile Red in the absence of *E. coli*, *C. elegans* feeding on the Nile Red *E. coli* mixture incorporated the dye specifically into lipid droplets within intestinal cells. No adverse effects on *C. elegans* growth rate, brood-size, pharyngeal pumping, dauer formation, dauer recovery, or lifespan was observed at Nile Red concentrations between 0.001  $\mu$ g/ml and 25  $\mu$ g/ml. The growth rate, brood-size, pharyngeal pumping, dauer formation, dauer recovery, and lifespan assays were carried out as follows. The mutant nematodes were compared to wild-type control nematodes.

To assay growth rate, nematode eggs were hatched in M9 buffer for twelve hours to obtain synchronized L1 progeny. The L1 progeny were then transferred to NGM/OP50 plates and maintained at 25°C. The period of time required for the population to reach the L4 stage was recorded.

To assay brood size, at least three L4 hermaphrodites were transferred to individual NGM/OP50 plates and allowed to lay eggs for forty-eight hours. The parents were then removed

and the number of progeny on each plate was scored within twenty-four hours (prior to the time at which the F1 progeny reached the egg-laying adult stage). All experiments were done at 25°C.

To assay pharyngeal pumping, nematodes are placed on NGM plates with OP50 bacteria 5 at 25° C, and observed under a dissection scope. The number of times the terminal bulb of the pharynx opens and closes per minute in the presence of bacteria is then determined.

Dauer formation, dauer recovery, and life span were assayed using standard methods known to the skilled artisan, and described, for example, in Malone, et al. (*Genetics* 143, 1193-1205, 1996).

10 To assay lifespan, nematodes were grown on NGM plates with OP50 bacteria at 25°C until the L4 stage (t=0). At least 50 nematodes were then transferred to NGM/OP50 plates containing 0.1 mg/ml 5-fluorodeoxyuridine to prevent growth of progeny. Nematodes were then observed every one to two days, and scored as dead when no longer responsive to gentle prodding with a platinum wire.

15 The specificity of Nile Red staining was confirmed using Sudan Black B, a dye that had previously been shown to specifically stain *C. elegans* fat (Sze et al., *Nature* 403:560-4, 2000; Wolkow, *Science* 290:147-50, 2000). For Sudan Black B staining, L4 or young adult nematodes were grown at 25°C and fixed in 1% paraformaldehyde. While in fixative, the nematodes were subjected to three freeze-thaws in dry-ice/ethanol. The nematodes were then incubated on ice for 20 ten minutes. The fixed nematodes were washed and dehydrated through a 25%, 50%, and 70% ethanol series of baths. The fixed and dehydrated nematodes were then stained in a saturated Sudan Black B solution (in 70% ethanol) for four hours.

Nile Red staining specificity was also confirmed by feeding nematodes BODIPY-labeled fatty acids (Figure 2A). C<sub>1</sub>-BODIPY 500/510 C<sub>12</sub> ( 4,4-difluoro-5-methyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoic acid), and C<sub>8</sub>-BODIPY 500/510 C<sub>5</sub> ( 4,4-difluoro-5-octoyl-4-bora-3a,4a-diaza-s-indacene-3-pentanoic acid) were purchased from Molecular Probes (D-3823 and D-3825). 1 mg/ml stock solutions of the BODIPY-labeled fatty acids were made in dimethyl sulfoxide (DMSO). The stocks were diluted 1:10,000 or 1:100,000 in PBS containing 20 μM bovine serum albumin. The diluted BODIPY-labeled fatty acid solutions were added to plates 30 containing lawns of *E. coli* OP50. Nematodes were placed on these plates as eggs or L1s and the

incorporation of BODIPY-labelled fatty acids was assessed in L4 or non-starved young adult nematodes. The incorporated fatty acids were visualized using UV fluorescence, and found to co-localize with Sudan Black staining (Figures 2B, 2C, 2D, 3A, and 3B).

## 5 Nile Red Staining Detects Quantitative Differences in Fat Accumulation

To determine whether Nile Red staining could be used to detect differences in nematode body fat accumulation, previously identified mutant nematodes having defects in fat accumulation were stained with Nile Red, as described herein.

Nile Red staining revealed increased fat stores in serotonin-deficient *tph-1(mg280)* 10 mutant nematodes when compared to wild-type nematodes (Figures 4A and 4B). Nematodes containing a deletion in the *tub-1* locus, the nematode ortholog of the murine mutant *tubby*, also displayed increased Nile Red staining (Figure 4C). Nile Red was also used to stain *C. elegans* *daf-2(e1370); daf-2(e1370); daf-16(mgDf37); and daf-2(e1370); daf-3(mgDf90)* mutant 15 nematodes (Figures 5A-5D). The number and pixel intensities of Nile Red staining droplets were quantitated using an imaging quantitation software package. All images were captured 20 using the Openlab software (Improvision Inc. Lexington, MA) on a Zeiss Axioplan II microscope equipped with rhodamine (emission 560-590 nm) and FITC/GFP (emission 500-515 nm) filters and a digital CCD camera (Hamamatsu C4742-95-12ER). All Nile Red images were acquired using equal exposure times such that the maximum pixel intensity of any image would 25 be below the maximal intensity recordable by the CCD camera. To quantitate pixel intensities and total pixel numbers, equal planes and regions of the nematode body were selected and the selection tool was used to shrink the region of interest to include only Nile Red Staining droplets. The total fluorescence of a selected area was calculated as the product of area multiplied by the mean fluorescence. At least three nematodes were quantitated for each condition and their 30 average was recorded. Similar results were found in multiple independent experiments. For each independent experiment, nematodes were compared to the reference N2 strain grown, stained, and then photographed under the exact same conditions.

Quantitation of Nile Red staining in wild-type and mutant nematodes is shown in Figure 5E. Nematodes with a deletion in a key biosynthetic serotonin enzyme, *tph-1(mg280)* (Sze, et al., *Nature* 403:560-4, 2000), or in the *tubby* homolog, *tub-1(nr2004)II* (kindly provided by Carl

D. Johnson), exhibited increased fat content detectable by Nile Red. *tub-1(nr2004)* mutant nematodes have ~2 fold increased fat content while *tph-1(mg280)* mutant nematodes accumulate ~2.5 fold greater fat levels than wild-type nematodes (Figure 5E). These *C. elegans* fat phenotypes are reminiscent of what has been seen in mammals, where serotonin and tubby signalling pathways affect body fat. Mice deficient either in *Tubby*, a novel gene product, or in *HTR2C*, a serotonin receptor, are obese (Noben-Trauth, et al., *Nature* 380:534-8, 1996; Tecott, et al., *Nature* 374:542-6, 1995).

Visualization of fat droplets by BODIPY-labeled fat compounds in *tph-1 (mg280)*, *tub-1 (nr2004)*, insulin, or TGF- $\beta$  pathway mutant nematodes closely matched the patterns and intensities observed by Nile Red staining.

Temperature-sensitive *daf-2(e1370)* mutant nematodes, containing a mutation in the insulin-like receptor, *DAF-2*, form dauers at the restrictive temperature (25°C) (Figures 6A and 6C). *daf-2(e1370)* were grown at the permissive temperature (15°C) past the developmental stage at which the shift to the alternative dauer developmental stage is initiated. The non-dauer *daf-2(e1370)* nematodes were then shifted to the non-permissive temperature. These temperature-shifted *daf-2(e1370)* non-dauers showed increased fat accumulation when stained with Nile Red (Figure 6B and 6D). The average number and pixel intensities of Nile Red staining fat droplets were calculated as described herein. *daf-2(e1370)* and *daf-7(e1372)* *C. elegans* staining is increased nearly 3-fold compared to wild-type *C. elegans*.

This result was consistent with results by Ogg et al. and Wolkow et al. showing that under these conditions *daf-2(e1370)* grow to be long-lived adults with increased fat stores (Ogg et al., *Nature* 389:994-9, 1997; Wolkow et al., *Science* 290:147-50, 2000).

Nematodes containing mutations in genes encoding components of the insulin-like and TGF- $\beta$  signaling pathways were also stained with Nile Red. Genetic analysis had previously shown that *daf-16(mgDf47)* suppressed the dauer and longevity phenotypes of *daf-2(e1370)*. Nile Red staining of *daf-16(mgDf47)* *daf-2(e1370)* double mutant nematodes revealed that *daf-16* also suppressed the *daf-2(e1370)* fat accumulation phenotype. Genetic analysis had previously shown that mutations in the TGF- $\beta$  pathway, such as *DAF-1*, a type I receptor, or *DAF-3*, a SMAD like transcription factor, fail to suppress *daf-2(e1370)* dauer and longevity phenotypes. Nile Red staining of *daf-2(e1370)*; *daf-1(m40)* or *daf-2(e1370)*; *daf-3(mgDf90)*

double mutant nematodes also failed to suppress the *daf-2(e1370)* fat accumulation phenotype. Thus, previously characterized epistatic relationships among the components of these signaling pathways were recapitulated by Nile Red fat staining (Figures 5A-5D).

Differences in body fat as visualized by Nile Red were correlated with actual fat content  
5 (Figure 7). Total lipids were extracted from wild-type, *tph-1(mg280)*, *daf-2(e1370)*, *daf-2(e1370); daf-16(mgDf47)*, *daf-16(mgDf47)*, *tph-1(mg280); daf-16 (mgDf47)* nematodes as follows. L1 synchronized nematodes were grown on four 6-cm plates at 15°C past the L2 stage of development, and then shifted to 25°C. Non-starved, young adult nematodes were washed off the plates with water, and placed into 15 ml polypropylene screw-capped centrifuge tubes. The  
10 tubes were spun at 1,000 x g for 1 minute. The pelleted nematodes were washed five times with water and then, after the final wash, the water was removed.

A 100 mg nematode pellet was required for each assay. Each pellet was split into two equal portions. Each portion was flash frozen in dry ice/ethanol and maintained at -80°C degrees until analyzed. One portion of each sample was used for fatty acid extraction while the  
15 other portion was used for protein extraction as described by Watts and Browse (Watts et al., *Arch. Biochem. Biophys.* 362:175-82, 1999; Watts et al., *Proc Natl Acad Sci USA* 99:5854-9, 2002). Briefly, after extraction and transmethylation of total lipids, phospholipids were separated on Thin Layer Chromatography plates (TLC) using chloroform:methanol: acetic acid (25:15:4). Triacylglycerides, diacylglycerides and phospholipids were separated from each other  
20 on TLC plates using hexane:diethyl ether:acetic acid (75:25:2). Separated samples were then analyzed by Gas Chromatography using an Agilent 6890 series machine equipped with a 30X 0.25-mm SP-2380 column (Supelco) (Watts et al., *Arch. Biochem. Biophys.* 362:175-82, 1999; Watts et al., *Proc. Natl. Acad. Sci. USA* 99:5854-9, 2002).

As expected, *tph-1(mg280)* and *daf-2(e1370)* nematodes have greater total fat content  
25 than wild-type nematodes (Figure 7). Moreover, as in mammals, the *daf-2(e1370)* excess fat is generally stored as triacylglycerides (Figure 7).

### Nile Red Staining Detected Fat Mobilization

Mobilization of fat stores in response to starvation can be monitored by Nile Red  
30 staining. When grown on Nile Red plus bacterial food plates, larval stage or adult wild-type

nematodes increase their fat content as they near starvation. When maintained in the starved state, they then undergo a progressive loss of Nile Red stained fat droplets. Furthermore, the loss of Nile red stained fat droplets is reversible when food is reintroduced to starved nematodes. *daf-2(e1370)*, *tph-1(mg280)*, or *tub-1(nr2004)* nematodes undergo similar alterations in fat

5 content in response to starvation.

Nile Red staining can also be used to detect the mobilization of fat droplets in response to fat mobilizing agents, for example, 5-aminoimidazole-4-carbozamide ribonucleoside (AICAR). AICAR is an adenosine analog that activates AMP-activated protein kinase (AMPK), a putative cellular energy sensor and metabolic master switch. Activation of AMPK results in the 10 mobilization of fat stores (Hardie et al., *Eur. J. Biochem.* 246:259-73, 1997; Hardie et al., *Ann. Rev. Biochem.* 37:821-55, 1998; Aschenbach et al., *Diabetes* 51:567-73, 2002; Corton et al., *Eur. J. Biochem.* 229:558-65, 1995).

Adult wild-type nematodes, *daf-2 (e1370)*, *tph-1(mg280)*, and *tub-1(nr2004)* mutant 15 nematodes were treated with 500 µM AICAR, and their fat content was detected using Nile Red as described above. Wild-type nematodes (Figures 8A and 8B), *daf-2 (e1370)* (Figures 8D and 8E), *tph-1(mg280)*, and *tub-1(nr2004)* mutant nematodes all displayed a progressive reduction in fat content relative to untreated control nematodes, and this reduction in fat content was detectable within twenty-four hours. To determine whether the effects of AICAR were 20 reversible, AICAR-treated nematodes were then moved to culture plates without AICAR. Without continued AICAR treatment, these nematodes regained their fat content. The effect of continuous AICAR treatment was tested by seeding AICAR plates with five hermaphrodites, and then monitoring the effect of AICAR on the growth and development of nematode progeny 25 relative to untreated control progeny. Continuous AICAR treatment resulted in a dramatic retardation of larval growth accompanied by depleted fat stores. These results indicated that Nile Red staining provides a sensitive, reproducible, and convenient method for assaying the body fat of living *C. elegans*. The result of AICAR treatment was also compared to the effect of starvation on wild-type (Figure 8A-8C) and *daf-2(e1370)* mutant nematodes (Figures 8D-8F). This comparison showed that the mobilization of fat stores in response to starvation or treatment with fat mobilizing agents can be monitored using Nile Red staining.

## Genetic Screen for Fat Mutant Nematodes

To identify mutant nematodes that display perturbations in fat content, droplet size, or localization, wild-type (N2) *C. elegans* were mutagenized with ethylmethanesulfonate (Brenner, *Genetics* 77:71-94, 1974). Synchronized populations of F1 and F2 progeny were then grown on 5 Nile Red plates and examined for their fat staining.

Mutagenesis screens were conducted on plates containing 0.005 µg/ml Nile Red. The screen and subsequent manipulations were conducted at 25°C (to reduce the number of fat accumulating *daf-2* mutant nematodes present in the F1 and F2 progeny).

*C. elegans* were placed on Nile Red plates either as eggs or starved L1s, and their Nile 10 Red staining phenotype was assessed when they reached the L4 stage and/or the young adult stage, prior to starvation, unless specified. Nile Red fluorescence was visualized under a Zeiss SV11 M2-bio microscope equipped with a rhodamine filter (emission 565-590 nm). For studying the impact of starvation on fat content, nematodes were grown to the L4 or young adult 15 stage on Nile Red plates. They were washed five time in M9 media and maintained either in M9 media or transferred to NGM plates without OP50. They were then monitored as described herein for their fat content.

The recovered nematode mutants defined several distinct classes: i) nematodes with increased fat, ii) nematodes with decreased fat, iii) nematodes with altered fat droplet morphology, and iv) nematodes with grossly distorted fat deposits accompanied by an altered 20 Nile Red emission profile. These mutant nematodes displayed a variety of lipophilic dye staining phenotypes (Figures 9A-9G) and thus were designated as *lpo* mutants. The Nile Red staining phenotype of each mutant was confirmed by BODIPY labeled fatty acid visualization and Sudan Black B staining. BODIPY-labeled fatty acids and Nile Red staining results in wild-type nematodes (Figure 10A and 10B), *lpo-1* (Figures 10C and 10D) and *lpo-2* (Figures 10E and 25 10F) mutant nematodes are shown.

The fat contents of *tph-1(mg280)*, *tub-1(nr2004)*, *daf-2(e1370)*, *lpo-1*, and *lpo-6* mutant nematodes were assayed by Nile Red staining. The results of this staining are shown in Table 1.

**Table I. Fat Content Assayed by Nile Red**

<u>STRAIN</u>	<u>STRAIN DESCRIPTION</u>	<u>FAT CONTENT ASSAYED BY NILE RED STAINING</u>
Wild-type		wild-type
<i>tph-1(mg280)</i>	deletion in serotonin biosynthetic enzyme	increased fat compared to wild-type
<i>tub-1(nr2004)</i>	deletion of nematode tubby locus	increased fat compared to wild-type
<i>daf-2(e1370)</i>	insulin receptor mutant	increased fat compared to wild-type
<i>lpo-1</i>	VLDL receptor mutant	increased fat compared to wild-type
<i>lpo-6</i>	identity of molecular lesion unknown	increased fat compared to wild-type and enlarged fat droplets

*lpo-1*, *lpo-2*, *lpo-3*, *lpo-4*, *lpo-5*, *lpo-6*, and *lpo-7* were each back crossed four times to wild-type (N2) nematodes. Standard genetic techniques were used to determine that *lpo-1*, *lpo-2*,

5 *lpo-3*, *lpo-4*, *lpo-5*, *lpo-6*, and *lpo-7* represented distinct complementation groups. All of the lpo mutations were recessive with fully penetrant phenotypes. While the fat staining patterns were most dramatic in adult nematodes, the phenotypes were also detectable at all larval stages. The characterization of *lpo-1* through *lpo-7* is summarized in Table II.

10

**Table II. Characterization of *LPO* mutant nematodes**

	<i>lpo-1</i>	<i>Lpo-2</i>	<i>lpo-3</i>	<i>lpo-4</i>	<i>lpo-5</i>	<i>lpo-6</i>	<i>lpo-7</i>
Fat content relative to wild-type	Increased	Reduced	Reduced	Reduced (green)	Reduced (green)	Enlarged Droplets	Reduced
Growth rate	115% of wt	Wt	wt	wt	wt	75% of wt	50% of wt
Adult body Length	wt	Wt	wt	wt	wt	wt	wt
Adult body Width	wt	Wt	wt	wt	wt	20% smaller than wt	wt
Brood size	wt	Wt	wt	wt	wt	less than wt	Much less than wt
Lifespan	wt	Wt	wt	wt	wt	1.7 fold greater than wt	wt
Survival rate when starved	120% of wt	30% of wt	wt	wt	wt	30% of wt	10% of wt
Dye filling	wt	Wt	wt	wt	wt	wt	wt

Table Legend

wt=wild-type

As indicated in Table II, *lpo-1* nematodes had a slightly increased growth rate (~15% faster than wild-type nematodes). In contrast, *lpo-6* nematodes, which have enlarged fat droplets, 15 exhibited a reduced growth rate (~25% slower than wild-type).

All *lpo* mutant nematodes were able to form dauers, recovered from the dauer stage, and developed into reproductive adults. Response to starvation was assayed as follows. Eggs were hatched in M9 buffer and equal numbers of nematodes (~100) were aliquoted into individual test tubes containing 1 ml of M9 media. Twice daily, during the first forty-eight hours after transfer 5 to M9, and once a day during for next eight days, nematodes in each aliquot were plated on NMG plates with lawns of OP50. Forty-eight hours after transfer to plates, the number of viable nematodes was scored. The reported results in Table II were the average of two independent experiments.

The reduced fat mutant nematodes, *lpo-2* and *lpo-3*, accumulated fat when they formed 10 dauers. Inspite of this increase, *lpo-2* and *lpo-3* dauer failed to accumulate the fat levels typical of wild-type dauers. When high fat *lpo-1* mutant nematodes formed dauers, *lpo-1* dauer had increased fat levels as compared to wild-type dauers.

These results indicate the *lpo* mutant nematodes may be used to identify genes that encode polypeptides that regulate feeding and metabolism (e.g., neuropeptides), hormonal 15 response pathways, subcellular fat trafficking, or fat droplet mobilization.

### ***lpo-1* Cloning**

For mapping each mutant, the mutant nematode was crossed to *C. elegans* strain CB4856. F2 hermaphrodite progeny of the cross, displaying the appropriate Nile Red phenotype (e.g. 20 increased staining for *lpo-1*, reduced staining for *lpo-3*), were then picked onto individual Nile Red plates, and allowed to self-fertilize. The Nile Red staining phenotype of F3 progeny was checked to ensure that the recombinants had been accurately picked. Once plates starved out, several hundred nematodes from each plate were pooled and their DNA was extracted as described by Williams (*Methods Cell. Biol.* 48:81-96, 1995). Using snip-SNP mapping (Wicks 25 et al., *Nat Genet* 28:160-4, 2001) the mutant loci were assigned chromosomal positions.

The *lpo-1* mutation was mapped using single nucleotide polymorphisms (SNP). The *lpo-1* mutation was generated in an N2-Bristol parental strain. This *lpo-1* mutant was then crossed with Hawaiian strain CB4856, a highly polymorphic *C. elegans* strain. *lpo-1* was mapped to a genomic region covered by the cosmid T13C2 as shown in Table III.

**Table III *lpo-1* SNP mapping**

(A) Chromosomal Linkage

Chromosome	I	II	III	IV	V	X
SNP clone	T22A3	T13C2	F10E9	C09G12	AC3	F45E1
allele	pkP1075	pkP2107	pkP3049	pkP4032	pkP5064	PkP6110
map position	+4.47	+0.08	-0.32	-3.64	+2.53	-0.83
# F2 recombinants with <i>lpo-1</i> phenotype tested	32	32	15	30	33	32
% CB4856	75%	0%	73%	86%	82%	82%

5 (B) Mapping within Chromosome II

SNP clone	C16C8	C01F1	T13C2	ZK666
allele	pkP2115	pkP2051	pkP2107	pkP2070
map position	-6.47	-3.95	+0.08	+2.57
# F2 recombinants with <i>lpo-1</i> phenotype tested	80	80	558	80
% CB4856	14%	10%	0%	10%
# homozygous wild-type F2 recombinants tested	0	0	51	0
% CB4856			100%	

Table IIIA shows that *lpo-1* was mapped to chromosome II. Table IIIB shows that *lpo-1* was mapped to a region of chromosome II.

After *lpo-1* was mapped to cosmid T13C2, the open reading frames on that cosmid were analyzed for likely *lpo-1* candidates. An open reading frame, T13C2.6, which encodes Very Low Density Lipoprotein (VLDL) receptor, was selected for further analysis. PCR primers were designed to amplify not only T13C2.6, but also several kilobases of flanking DNA. The nucleic acid sequence of the forward and reverse primers, respectively, were  
10 5'CACAACAAGTCAGCAAGCAATACAAGTGG 3' (SEQ ID NO: 7) and  
15 5' GTAGGAGATGTGACCAATCGTTGAAGTG (SEQ ID NO:8). The purified 9.5-kb PCR fragment consisting of the complete T13C2.6 coding sequence, and 2604 basepairs (bp) upstream and 2674 base pairs of downstream sequence was injected into *lpo-1* nematodes at 1.5, 3, 5, and 15 ng/ $\mu$ l, in combination with a nucleic acid encoding a visible marker, *SUR-5::GFP*

(Yochem et al., *Genetics* 149:1323-34, 1998) at 80 ng/μl. Stable lines were maintained by picking green nematodes. No other predicted full length or partial ORFs were contained in this 9.5 kb fragment.

The T13C2.6 containing fragment rescued the *lpo-1* fat phenotype (Figures 11A and  
5 11B).

### ***lpo-1* Encodes a VLDL Receptor Homolog**

*lpo-1* encodes a predicted 863 amino acid protein that has 35% amino acid sequence identity to human (SEQ ID NO:9) and rat (SEQ ID NO:10) very low density lipoprotein (VLDL) receptors. An alignment of the *C. elegans*, human, and rat VLDL amino acid sequences is shown in Figure 11G. Mammalian VLDL receptors contain eight adjacent LDL type A domains, which are ligand binding repeats stabilized by three cysteine disulfide bonds. The A domains are followed by an epidermal growth factor (EGF) homology domain, modules of type B LDL repeats (including the YWTD consensus tetrapeptide), a single pass transmembrane domain, and 15 a short cytosolic tail. The amino acid sequence, NPXY, which is thought to be the receptor internalization signal, resides in the VLDL receptor's short cytosolic tail (Brown et al., *Nature* 388:629-30, 1997; Nimpf et al., *Atherosclerosis* 141:191-202, 1998; Trommsdorff et al., *Cell* 97:689-701, 1999). The predicted topology of LPO-1 closely matches the described domain structure of VLDL-receptors (Figure 11C). The *lpo-1* genomic nucleic acid sequence (SEQ ID 20 NO:1) is shown in Figure 11D. The nucleic acid sequence (SEQ ID NO:2) of the *lpo-1* open reading frame is shown in Figure 11E. The LPO-1 amino acid sequence (SEQ ID NO:3) is shown in Figure 11F.

The LDL receptor gene family comprises five mammalian and several invertebrate members that are predicted to mediate extracellular ligand endocytosis. The role of LDL 25 receptors in cholesterol endocytosis, trafficking, and homeostasis is well established. The major structural difference between mammalian LDL and VLDL receptors is the number of ligand binding repeats. LDL receptors contain seven type A repeats, while VLDL receptors contain eight type A repeats (Nimpf et al., *Atherosclerosis* 141:191-202, 1998). Chickens lacking VLDL receptor are obese and sterile, due to defective yolk deposition. In contrast, mice lacking the 30 VLDL receptor do not gain excess weight and appear to be protected from obesity (Frykman et

al., *Proc. Natl. Acad. Sci. USA* 92:8453-7, 1995; Tacken et al., *Curr. Opin. Lipidol.* 12:275-9, 2001). Interestingly, mice lacking two LDLR superfamily members, VLDLR and ApoE receptor 2 (ApoER2) have neuronal migration defects (Trommsdorff et al., *Cell* 97:689-701, 1999). VLDLR and ApoER2 are predicted to be cell surface receptors for Reelin, a guidance molecule 5 associated with neuronal migration (Trommsdorff et al., *Cell* 97:689-701, 1999).

In light of the yolk abnormalities observed in VLDL-receptor deficient chickens, vitellogenin deposit was examined in *lpo-1* mutant nematodes. Vitellogenins are yolk proteins that are synthesized and secreted by nematode intestinal cells. RME-2, a member of the LDL receptor superfamily, is predicted to be the egg yolk receptor that mediates yolk deposit in *C. elegans* (Grant et al., *Nat. Cell. Biol.* 3:573-9, 2001; Lin et al., *Nat. Cell. Biol.* 3:567-72, 2001). This prediction is based on the observation that vitellogenin:GFP (YP170::GFP) accumulated in eggs of wild-type nematodes, but was mislocalized in *rme-2* mutant nematodes (Grant et al., *Nat. Cell Biol.* 3:573-9, 2001; Lin et al., *Nat. Cell Biol.* 3:567-72, 2001). The YP170::GFP reporter construct was crossed into *lpo-1* mutant nematodes, and used to study egg yolk accumulation.

15 The localization of YP170::GFP in *lpo-1* mutant nematodes was indistinguishable from that observed in wild-type nematodes. Thus, *LPO-1* did not affect egg yolk receptor-mediated endocytosis.

Next, inhibitors of HMG-CoA reductase (e.g., lovastatin) were tested to assess whether they affected the fat content of *lpo-1* mutant nematodes. Hydroxymethylglutaryl-CoA (HMG-20 CoA) reductase is an enzyme that functions in a key regulatory step of cholesterol biosynthesis. Inhibitors of HMG-CoA reductase have been commonly used to lower human cholesterol levels. The high fat content of *lpo-1* mutant nematodes was reduced when they were grown on plates containing 200 µg/ml mevinolin, an HMG-CoA reductase inhibitor.

### 25 *lpo-3* Cloning

Another exemplary gene identified by the method described above, termed *lpo-3*, was also mapped and cloned. Genetic mapping using SNP polymorphisms placed *lpo-3* between cosmids C18E3 and K04F10 on chromosome I as shown in Table IV.

**Table IV. *lpo-3* SNP mapping**

(A) Chromosomal designation

Chromosome	I	II	III	IV	V	X
SNP clone	T22A3	T13C2	F10E9	C09G12	AC3	F45E1
allele	pkP1075	pkP2107	pkP3049	pkP4032	pkP5064	PkP6110
map position	+4.47	+0.08	-0.32	-3.64	+2.53	-0.83
# F2 recombinants with <i>lpo-3</i> phenotype tested	40	38	39	35	40	38
% CB4856	15%	76%	72%	85%	83%	71%

5 (B) Chromosome I mapping

SNP clone	C18E3	C09D4	T22A3	ZK1025
allele	pkP2115	PkP1055	PkP1075	PkP1066
map position	-1.49	+0.09	+4.74	+8.84
# F2 recombinants with <i>lpo-3</i> phenotype tested	247	48	254	48
% CB4856	5.6%	0%	9.4%	19%

(C) Establishment of mapping boundaries

SNP: C18E3 C09D4 K04F10 D2030 T23G11 F18C12 VF3H21 Y67A6A T22A3

Map: (-1.49) (+0.09) (+0.92) (+2.12) (+2.23) (+2.46) (+2.94) (+3.99) (+4.78)

10 recombinants

#1	N/N	N/N	N/N	N/C	N/C	N/C	N/C	N/C	N/C
#6	N/N	N/N	N/N	N/N	N/C	N/C	N/C	N/C	N/C
#8	N/N	N/N	N/N	N/C	N/C	N/C	N/C	N/C	N/C
#17	N/N	N/N	N/N	N/N	N/N	N/C	N/C	N/C	N/C
15 #24	N/C	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
#39	N/C	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
#121	N/N	N/N	N/C	N/C	N/C	N/C	N/C	N/C	N/C
#127	N/N	N/N	----	----	N/N	N/N	N/N	N/N	N/C
20 #132	N/N	N/N	----	----	N/N	N/N	N/C	N/C	N/C
#156	N/N	N/N	----	----	N/C	N/C	N/C	N/C	N/C
#167	N/C	N/N	----	----	N/N	N/N	N/N	N/N	N/N
#176	N/C	N/N	----	----	N/N	N/N	N/N	N/N	N/N
#209	N/C	N/N	----	----	N/N	N/N	N/N	N/N	N/N
25 #244	N/N	N/N	----	----	N/C	N/C	N/C	N/C	N/C

Table Legend

N: N2 Bristol, wild-type chromosomal SNP detected

C: CB4856 recombinant SNP detected

Table IVA shows that *lpo-3* was mapped to chromosome I. Table IVB shows that *lpo-3* was mapped to a specific region of chromosome I. Table IVC shows that mapping boundaries were established using F2 recombinant progeny (of an *lpo-3* mutant nematode crossed to *C. elegans* strain CB4856) that displayed an *lpo-3* mutant phenotype.

Having mapped *lpo-3* to a relatively small interval, a set of RNAi-expressing bacteria was screened. These bacteria were described by Fraser et al. and Kamath et al (Fraser et al., *Nature* 408:325-30, 2000; Kamath et al., *Genome Biol.* 2:RESEARCH0002). The nematodes cultured on these RNAi-expressing bacteria were then analysed using Nile Red fat staining, as described herein. This analysis identified C34G6.4 as an *lpo-3* candidate. When wild-type nematodes were fed C34G6.4 RNAi-expressing bacteria, they mimicked the low fat phenotype of *lpo-3*. To test whether C34G6.4 could rescue the *lpo-3* phenotype, a genomic region flanking the open reading frame was amplified by PCR. 10 kb and 7.8 kb PCR fragments (with 1 kb overlap) were used to cover the entire C34G6.4 coding sequence, including 9,051 base pairs of upstream and 1,367 base pairs of downstream sequence. The 10 kb fragment contained the upstream sequences plus 1,244 base pairs of C34G6.4 coding sequence, while the 7.8 kb fragment contained 300 nucleotides downstream of the C34G6.4 ATG codon, and extended to 1,367 base pairs downstream of the C34G6.4 stop codon. *lpo-3* mutant nematodes injected with these overlapping PCR fragments displayed wild-type fat staining (Figures 12A and 12B) only when both fragments were injected into *lpo-3* mutant nematodes at 2.5, 5, or 7.5 ng/μl. No rescue was noted when the PCR fragments were injected individually into *lpo-3* mutant nematodes at 5 ng/μl. (These injections were carried out using the visible co-injection marker *SUR-5::GFP* (80 ng/μl)). The topology of the predicted LPO-3 protein is shown in Figure 12C.

This result suggested that C34G6.4 was *lpo-3*. No other predicted full length or partial ORFs were contained in the two overlapping PCR fragments. Sequencing of the C34G6.4 genomic fragment identified a G to A transition in exon 12, which caused a Glycine (G) to Aspartic acid (D) change at position 1163 of the predicted protein. Thus *lpo-3* is C34G6.4. The genomic nucleic acid sequence (SEQ ID NO:4) of *lpo-3* is shown in Figure 12D. The *lpo-3* open reading frame is shown in Figure 12E. The amino acid sequence of LPO-3 is shown in Figure 12F.

### *lpo-3* Encodes an ABC-Type Transporter Homolog

*lpo-3* encodes a predicted protein of 1,265 amino acids that is a P-glycoprotein family member with 45% and 44% amino acid identity to human (SEQ ID NO:11) and mouse (SEQ ID NO:12) ATP-Binding Cassette (ABC)-type transporters, respectively. An alignment of the *C.*

5 *elegans*, mouse and human ABC transporter proteins is shown in Figure 12G. In humans, ABC-type transporter molecular lesions cause cholesterol and lipid homeostasis disorders, such as Tangier disease, familial HDL deficiency, progressive familial intrahepatic cholestasis type 2 and type 3, adrenoleukodystrophy, and sitosterolaemia (Ioannou, *Nat. Rev. Mol. Cell Biol.* 2:657-68, 2001). At least ten ABC-type transporters have previously been shown to facilitate  
10 cholesterol and lipid flux across membrane bilayers (Geourjon et al., *Trends Biochem. Sci.* 26:539-44, 2001; Holland et al., *J. Mol. Biol.* 293:381-99, 1999). *lpo-3* contains a pair of ATP-binding domains and two sets of transmembrane domains that are characteristic of ABC-type transporters. A comparison of the *lpo-3* encoded protein with other ABC-type transporters indicated that the G to D mutation occurred in a functionally important conserved LSGQQ  
15 nucleotide binding domain (Geourjon et al., *Trends Biochem. Sci.* 26:539-44, 2001; Holland et al., *J. Mol. Biol.* 293:381-99, 1999). These results indicated that *lpo-3* regulates fat dynamics in *C. elegans*. A schematic diagram depicting the structure of *lpo-3* is shown in Figure 11C.

The *C. elegans* genome contains three other P-glycoprotein family members: *pgp-1*, *pgp-3*, and *mrp-1*. To test whether the proteins encoded by these genes are involved in *C. elegans* fat  
20 dynamics, the following strains were obtained: *pgp-1(pk17)* IV, *pgp-3(pk18)* X, *mrp-1(pk89)* X; *pgp-1(pk17)* IV; *pgp-3(pk18)* X; *mrp-1(pk89)* (Broeks et al., *EMBO J.* 14:1858-66, 1995).

Three of these previously described nematode deletion mutants, *pgp-1(pk17)*, *pgp-3(pk18)*, and *mrp-1(pk89)*, were stained with Nile Red. Relative to wild-type nematodes, *pgp-3(pk18)* nematodes displayed slightly increased fat content; *pgp-1(pk17)* mutant nematodes  
25 displayed reduced fat content during adulthood, particularly when starved, but their fat content appeared wild-type during larval stages; and *mrp-1(pk89)* staining was indistinguishable from wild-type nematode staining. Nematodes having deletions in all three genes, *pgp-1(pk17)*; *pgp-3(pk18)*; *mrp-1(pk89)*, were viable and had normal fat content. Feeding-mediated RNAi was used to inactivate *lpo-3* in the individual P-glycoprotein deletion mutant nematodes as well as in  
30 the triple mutant (i.e., *pgp-1(pk17)*; *pgp-3(pk18)*; *mrp-1(pk89)*). Inactivation of *lpo-3* in the

individual P-glycoprotein mutant nematodes, *pgp-1*, *pgp-3*, *mrp-1*, or in the triple mutant, resulted in a low fat phenotype. Fat levels in these *lpo-3* RNAi mutant nematodes were comparable to those observed in the original *lpo-3* mutant nematode.

5       *lpo-1* and *lpo-3* therefore encode polypeptides whose human homologs are critical regulators of cholesterol and lipid homeostasis. Their identification in an unbiased screen for *C. elegans* fat metabolism regulators provides proof that the methods of the invention are useful not only for the identification of *C. elegans* fat metabolism regulator genes, but also for the identification of their human homologs. These working examples demonstrate that human fat/sterol disease genes can be identified and studied in *C. elegans* using the methods of the  
10      invention.

### **Feeding-Mediated RNAi Is Useful for Studying Fat Metabolism Regulator Genes**

In *C. elegans* many expressed genes are subject to inactivation by RNAi (Fire et al., *Nature* 391:806-11, 1998; Fraser et al., *Nature* 408:325-30, 2000). RNAi may be accomplished  
15      by growing *C. elegans* on plates of *E. coli* expressing double stranded RNA. The nematodes feed on RNA-expressing bacteria, and this feeding is sufficient to cause the inactivation of specific target genes (Fraser et al., *Nature* 408:325-30, 2000; Kamath et al., *Genome Biol* 2, 2001). To test whether RNAi feeding could be used to study fat metabolism regulator genes, high fat *daf-2(e1370)* nematodes were fed *daf-16* or *daf-12* RNA-expressing bacteria. These  
20      nematodes were then analyzed using Nile Red, as previously described. The RNAi assays were carried out as follows.

Bacteria containing each RNAi clone were cultured in 300 µl Luria Broth (LB) media containing 50 µg/ml ampicillin for six to fourteen hours. 40 µl of each culture was then spotted  
25      in a single well of a 24-well plate containing NGM agar, 6 mM IPTG, and 25 µg/ml carbenicillin. After overnight induction with IPTG, Nile Red was added to each well to a final concentration of 0.05 µg/ml. Five to ten synchronized L1 nematodes were then added to each well and incubated at 20°C. Growth conditions and Nile Red staining of nematodes were assessed after forty-eight, seventy-two, and ninety-six hours using light phase and UV fluorescence microscopy. For each batch of RNAi clones tested, L4440 (vector control) and  
30      OP50 control wells were included. At forty-eight hours, nematodes in control wells would be

expected to have reached the L4 or young adult stage. By seventy-two hours, nematodes in control wells were at or near starvation. A fat metabolism phenotype was assigned to an RNAi well only if a majority of the nematodes in that well displayed a fat metabolism phenotype. Wells producing a fat metabolism phenotype were then re-tested in at least two independent trials. In all cases, the fat metabolism phenotype was scored blind, i.e. the investigator was unaware of the identity of the target RNAi clone while scoring the phenotype.

Results with feeding-mediated RNAi paralleled previous results obtained with genetic analysis, showing that mutations in the forkhead transcription factor gene, *daf-16*, and in the nuclear hormone receptor gene, *daf-12*, suppressed *daf-2*'s high fat phenotype. These results indicated that feeding-mediated RNAi is useful for the analysis of fat metabolism regulator genes.

Feeding-mediated RNAi was then used to study *lpo-1* nematodes. *lpo-1* mutant nematodes were fed RNAi bacteria expressing *daf-16*, *daf-12*, or *daf-3*, and then the *lpo-1* mutant nematodes were analyzed using Nile Red, as described herein. While *daf-16* and *daf-12* suppressed the high fat phenotype of *daf-2*, *lpo-1* nematodes high fat phenotype remained unchanged when grown on *daf-16*, *daf-12*, or *daf-3* RNAi bacteria. This indicated that *lpo-1*'s high fat phenotype was not caused by defects in insulin or TGF- $\beta$  signaling.

### **Systematic Identification of Fat Metabolism Regulator Genes using RNAi**

To identify additional fat metabolism regulator genes, a double stranded RNAi bacterial library with a coverage of greater than 80% of the 19,000 known and predicted *C. elegans* ORFs was utilized (Fraser, et al., *Nature* 408:325-30, 2000; Kamath et al., *Genome Biol.* 2; 2001). The potency and specificity of this library was demonstrated by studies showing that 90% of genes identified by classical genetics as causing embryonic lethality when disrupted also cause embryonic lethality when inactivated by feeding RNAi (Fraser, et al., *Nature* 408:325-30, 2000). Combining the Nile Red fat content assay with the bacterial feeding RNAi strategy, a systematic analysis of genes that regulate fat content, fat droplet morphology, and pattern of fat droplet deposition was conducted.

For each targeted open reading frame, a plate of bacteria expressing the corresponding double stranded RNA was cultured. *C. elegans* L1 larva were then transferred to the plate and

allowed to feed on the RNAi-expressing bacteria. Nile Red was also present on the plate, as described herein. The fat phenotype of non-starved adults was then analyzed. RNAi clones producing an increased or reduced fat phenotype were confirmed by re-testing in at least 2 independent experiments. Of 16,757 genes tested by RNAi, 2% (325 genes) (Table V) (a) 5 caused reduced fat content or distorted fat deposition pattern, while 0.7% (116 genes) (Table VII) resulted in nematodes with increased fat content or enlarged fat droplet size. RNAi inactivation of another 240 genes produced reduced fat accompanied by larval arrest, embryonic lethality, or sterility (Table VI).

#### 10 **RNAi Clones that Reduce Fat without Interfering with Growth or Development**

This analysis identified three hundred twenty-five genes whose inactivation caused a reduction in fat content or fat mislocalization, but did not interfere with growth or development. These RNAi clones produced fat phenotypes that ranged from dramatic alterations in fat content or deposition pattern to more subtle changes. For the most part, fat droplets remained confined 15 to intestinal cells even in mutant nematodes in which the distinct rows of intestinal fat droplets were distorted (these mutant nematodes are designated as distorted in Table V, an example of this phenotype is shown in Figure 9G).

Inspection of the list of the genes whose inactivation caused altered fat content in fertile adults revealed a wide range of biological molecules including metabolic enzymes, signal 20 transduction factors, transcription factors, receptors, channels, transporters, adhesion molecules, vesicular transport molecules, structural proteins, general cellular maintenance components and a significant number of genes with previously uncharacterised functions (Tables V, VI and VII). Some of the genes on these lists are known to be key players in mammalian fat or lipid metabolism. For example, reduced levels of stored fat resulted from RNAi of genes encoding 25 nematode homologs of enzymatic components of membrane lipid biosynthetic machinery (such as choline/ethanolamine phosphotransferase, and CDP-alcohol phosphatidyltransferase),  $\beta$ -oxidation ( $\Delta 2$ ,  $\Delta 4$ , dienoylCoA reductase, 3-hydroxyacyl-CoA dehydrogenase, long chain acyl-CoA thioesterase), fatty acid elongation enzymes, and cytosolic fatty acid and acylCoA binding proteins. Similarly, reduced fat content or distorted deposits resulted from RNAi of several 30 known components of sterol metabolism, for example nematode homologs of HMG-CoA

reductase (catalyzing the conversion of hydroxymethylgutaryl-CoA to mevalonate, the committed step of cholesterol biosynthesis), SREBP, LCAT (Lecitin-cholesterol acyltransferase, a facilitator of reverse cholesterol transport and modifier of ApoB-containing lipoproteins), and Steroidogenic acute regulatory (StAR- responsible for the transport of cholesterol from the outer 5 to the inner mitochondrial membrane, the rate-limiting step in steroidogenesis) related protein (Figure 13).

RNAi inactivation of glyceraldehyde-3-phosphate-dehydrogenase (GAPDH, an insulin regulated glycolytic enzyme) and phosphoenolpyruvate carboxykinase ((PEPCK), an enzyme which catalyses a regulated step of gluconeogenesis in adipose tissue (Hanson, et al., *Ann. Rev. 10 Biochem.* 66:581-611, 1997)) reduced body fat content. GAPDH mRNA upregulation is associated with fat storage and lipogenesis in adipocytes of obese Zucker rats (Rolland, et al., *J. Biol. Chem.* 270:1102-6, 1995). Similarly, PEPCK expression is upregulated in several animal models of obesity and type II diabetes (Friedman, et al., *J. Biol. Chem.* 272:31475-81, 1997; Yoon, et al., *Nature* 413:131-8, 2001), while selective down regulation of PEPCK expression in 15 mouse adipocytes correlates with reduced adipose tissue size and fat content (Olswang, et al., *Proc. Natl. Acad. Sci. USA* 99:625-30, 2002).

RNAi of *C. elegans* homologs of genes that function in gastrointestinal digestion and uptake of food in mammals also affect fat phenotype. In humans, PepT-1 is an insulin responsive transporter of dipeptide and tripeptides that is located in the intestinal brush border 20 and provides a mechanism for protein absorption (Adibi, *Gastroenterology* 113:332-40, 1997). RNAi down regulation of *C. elegans* *ptr-2* locus, the PepT-1 homolog, produced a reduction in fat content as did the inactivations of ZK6.7 and R07B7.9, which encode two lipases most similar to mammalian gastric and brush-border lipases, respectively.

In mammals, CNS control of satiety is fundamental to the regulation of mammalian 25 appetite and weight control. RNAi inactivation of a number of *C. elegans* genes that may function in food sensation and neuroendocrine signaling resulted in aberrant fat content. RNAi targeting of C43H6.9, for example, a putative glutamate receptor, and F56B6.5, a putative G-coupled protein with homology to rat hippocampal somatostatin receptor, lead to increased fat storage. Reduced fat content resulted from RNAi inactivation of R11A5.1, a homolog of 30 neuronal β-adaptin, H27A22.1, encoding the potential ortholog of glutaminyl cyclase (required

for biosynthesis of pyroglutamyl peptides), and several chemoreceptor and nematode olfactory receptors.

RNAi of specific nuclear hormone receptor genes produced nematodes with reduced or increased fat content. Nuclear hormone receptors regulate fat and sterol metabolism either by modulating transcription of metabolic genes or by initiating organelle or cellular differentiation cascades, notably peroxisomes and adipocytes, or by modulating transcription of sterol modifying and transport enzymes (Chawla, et al., *Science* 294:1866-70, 2001; Lazar, *Genes Dev.* 16:1-5, 2002; McKenna, et al., *Cell* 108:465-74, 2002; Willson, et al., *Ann. Rev. Biochem.* 70:341-67, 2001).

Although *C. elegans* lack dedicated adipocytes, their intestinal cells function as a major site of fat storage. Interestingly, several genes found to affect fat levels in *C. elegans* are homologs of mammalian proteins that function in adipocyte recruitment, growth, and differentiation. Adipocytes produce and secrete lysophosphatidic acid (LPA) among other peptidic and lipid factors. Paracrine regulation of preadipocyte growth is thought to be one of the biological activities mediated by LPA (Pages, et al., *Ann. NY Acad. Sci.* 905:159-64, 2000). G-protein coupled receptors (LPA<sub>1</sub>/EDG-2) have been identified as potential transducers of the LPA signal (Pages, et al., *Ann. NY Acad. Sci.* 905:159-64, 2000). Inactivation of nematode homolog of LPA<sub>R1</sub>/Edg-2 receptor results in reduced fat.

The reduced fat phenotypes were categorized as (i) much reduced (i.e., less than 20% of the fat content present in wild-type nematodes; fat content in these nematodes was reduced by at least 80%, 85%, 90%, 95%, 97%, 99% or 100%); (ii) reduced and/or distorted (i.e., less than 50% of the fat content present in wild-type nematodes; fat content in these nematodes was reduced by at least 50%, 55%, 60%, 70%, 75%, or 79%); (iii) moderately reduced (i.e., less than 75% of the fat content present in wild-type nematodes; fat content in these nematodes was reduced by at least 25%, 30%, 35%, 40%, 45%, or 49%); or (iv) slightly reduced (i.e., less than 97.5% of the fat content of wild-type nematodes; fat content in these nematodes was reduced by at least 2.5%, 5%, 10%, 15%, 20%, or 24%). The percentages of the 325 genes falling into each of these categories were 15%, 61%, 19%, and 5%, respectively. The list of target genes identified was annotated using BlastP searches against Genbank mammalian databases. This list identifies the target genes by *C. elegans* cosmid name and open reading frame number. In

addition, information available at nematodebase ([www.nematodebase.org](http://www.nematodebase.org)), a central repository of data on *C. elegans* was also used.

**Table V. RNAi Clones that Reduce Fat Content or Alter Fat Deposition Pattern without Reducing Viability**

5

<i>C. elegans</i> Gene	Brief Description	Nile Red Fat Phenotype
<b>METABOLIC ENZYME (38)</b>		
C36A4.9	acetyl-CoA synthetase	reduced
AH10.1	medium-chain acyl-CoA synthetase	reduced
C17C3.1	peroxisomal long-chain acyl-coA thioesterase	reduced
K05F1.3	acyl-coA dehydrogenase	distorted, reduced
T08B2.7	gastrin-binding/3-hydroxyacyl-Coenzyme A dehydrogenase	much reduced
W01C9.4	mitochondrial Δ2,Δ4-dienoyl-CoA reductase	distorted
T02G5.4	acyl-CoA thiolase	slightly reduced
F14H8.1	long chain fatty acyl elongase	reduced
F11E6.5	fatty acid elongase	reduced
B0285.8	choline/ethanolamine kinase	distorted, reduced
Y49A3A.1	choline/ethanolamine phosphotransferase	much reduced
F23H11.9	CDP-alcohol phosphatidyltransferase	moderately reduced
C01C10.3	phospholipid and glycerol acyltransferase	slightly reduced
F08F8.2	3-hydroxymethylglutaryl-CoA (HMG-CoA) reductase	reduced
F15A8.6	cholesterol esterase	reduced
K02D3.2	steroidogenic acute regulatory (StAR) related	distorted
M05B5.4	LCAT-like lysophospholipase	distorted
K10B3.7	glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	reduced
H04M03.1	phosphoenolpyruvate carboxykinase (PEPCK-C)	reduced
F43H9.2	serine palmitoyltransferase II	reduced
Y6B3B.10	lag1 (ceramide synthesis)	distorted
K09D9.2	cytochrome P450	much reduced
K07C6.4	cytochrome P450 2C2 (P450 PBC2)	moderately reduced
K07C6.5	cytochrome P450 2C2 (P450 PBC2)	much reduced
T04A8.16	calpain-type cysteine-protease	moderately reduced
F28H6.3	1-aminocyclopropane-1-carboxylic acid synthase	reduced
C06E7.3	S-adenosylmethionine synthetase	reduced
F13D11.1	lysosomal acid phosphatase precursor	much reduced
F52B11.2	phosphomannomutase 2	reduced
K03B8.3	neutral zinc metallopeptidases	distorted
C24A11.9	trans-prenyltransferase	reduced, slow growth
T09B4.8	alanine-glyoxylate aminotransferase 2	reduced
Y55F3C.c	putative thioredoxin	distorted, few droplets
T12A2.1	chlorohydrolase/histidine degradation	distorted
C31H2.3	4-hydroxyphenylpyruvate dioxygenase	moderately reduced
E01A2.i	glutamate-cysteine ligase/oxidative stress	reduced
C46H11.2	flavin binding monooxygenase	moderately reduced
M28.6	serine beta lactamase-like protein	reduced

FAT/LIPID interacting (8)		
F37B12.3	lipid associated protein	much reduced
C37H5.3	esterase/lipase	moderately reduced
ZK6.7	gastric lipase/esterase	reduced
R07B7.9	adult-specific brush border esterase/phospholipase	reduced
F31F6.7	lipase	reduced
F13D12.6	esterase/lipase/Serine carboxypeptidase (S10)	much reduced
C15B12.7	lipocalin and cytosolic fatty-acid binding protein	reduced
C44E4.6	acyl-coA-binding protein	reduced
TRANSCRIPTION FACTOR (19)		
Y47D3B.7	SREBP	distorted
C33G8.9	nuclear hormone receptor/C4-type steroid receptor	distorted
K08A2.b	nuclear hormone receptor/hepatocyte nuclear factor 4	much reduced
Y69A2A_7278.1	nuclear hormone receptor/hepatocyte nuclear factor 4	reduced, distorted
F11C1.6	steroidogenic factor 1	reduced
B0280.3	nhr-10 (ribose 5-phosphate ketol-isomerase)	distorted
F11A1.3	similarity to vitamin D (1,25-dihydroxyvitamin D3) receptor	reduced
C46E10.9	zinc finger, C2H2 type	moderately reduced
C47C12.3	zinc finger, C2H2 type/mouse OPR	much reduced
T09F3.1	zinc finger, C2H2 type	reduced
T23F11.4	zinc finger, C2H2 type	moderately reduced
ZK686.4	zinc finger, C2H2 type	reduced
Y116A8C.32	Zn-finger CCHC type transcription factor/ ZFM1	reduced
F22A3.4	homeotic protein PBX2 homology	reduced
C09G9.7	LuxR family/'Paired box' domain	slightly reduced
W02C12.3	microphthalmia transcription factor/Waardenburg syndrome	reduced
F22A3.5	pre-B-cell leukemia transcription factor 1 homology	moderately reduced
C01G6.5	Forkhead-associated (FHA) domain	reduced
F39D8.2	weak similarity to homeobox protein PKNOX	reduced
Translational control (5)		
R04A9.4	translation initiation factor 4E (eIF-4E)	moderately reduced
ZK757.3	translation initiation factor eIF-2C	reduced
Y41E3.10	elongation factor 1 beta/beta'/delta chain	much reduced
D2089.2	RNA-binding region RNP-1	much reduced
F11A10.3	zinc finger C-x8-C-x5-C-x3-H type	reduced
SIGNAL TRANSDUCTION (30)		
B0218.5	serine/threonine protein kinase	moderately reduced
T05C12.1	serine/threonine protein kinase	moderately reduced
Y53C12A.1	serine/threonine protein kinase/membrane associated	reduced
C16A11.3	serine/threonine protein kinase	moderately reduced
F45H7.4	pim1 serine/threonine-protein kinase	reduced
ZK930.1	G-protein beta WD-40 repeats-ser/thr protein kinase/PI-3	reduced
ZC504.4	tyrosine kinase and serine/threonine protein kinase	distorted
M01B12.5	tyrosine kinase catalytic domain	reduced
C02F4.2	serine/threonine protein phosphatase (PP2b)	moderately reduced
C06A1.3	serine/threonine specific protein phosphatase	slightly reduced
ZC302.1	serine/threonine specific protein phosphatase	distorted
C03D6.3	dual specificity protein phosphatase/RNA guanylytransferase	distorted

T19D2.2	dual specificity protein phosphatase family	reduced
C47D12.1	phosphatidylinositol 3- and 4-kinase/EF-hand family	reduced
ZK909.3	guanosine-3',5'-bis(diphosphate)-pyrophosphohydrolase	moderately reduced
C06A6.1	phosphotriesterase	moderately reduced
R107.4	IKK-related kinase epsilon	distorted
C33H5.17	D111/G-patch domain	reduced
R07E5.1	D111/G-patch domain	moderately distorted
C41D7.2	HMGCR/Patched 5TM box	distorted
ZK675.1	HMGCR/Patched 5TM box-patched	reduced
F20H11.2	mop-3/strawberry notch (sno)	reduced
T04D3.2	EF-hand family domain protein/no significant similarity	distorted
C44F1.5	guanylate cyclase	much reduced
H08M01.2	RhoGAP domain/glucocorticoid receptor	reduced
F46G11.3	protein kinase	reduced
K10D3.5	adaptor protein/nuclear receptor binding protein/kinase	moderately reduced
F41D9.1	RabGAP/TBC domain/SH3 domain	reduced
F07C3.4	RCC1 domain	distorted
F45E4.6	EGF-like domain/Crystallin	distorted
<b>RECEPTORS (22)</b>		
T14E8.3	dopamine receptor D2	much reduced
C07A9.2	G10 protein/edg-2/LPA receptor	reduced
Y4C6A.h	metabotropic glutamate receptor	distorted
C38C10.1	rhodopsin-like GPCR superfamily/neurkinin-3 receptor	reduced
C34C6.6	peroxisomal targeting signal 1 receptor	reduced
E02C12.3	rhodopsin-like GPCR superfamily	moderately reduced
H09F14.1	rhodopsin-like GPCR superfamily/somatostatin like	distorted
Y44A6B.2	rhodopsin-like GPCR superfamily	reduced
F58G4.2	chemoreceptor	distorted
Y40H7A.7	Sra family chemoreceptor	reduced
F07C4.1.	7-Helix G-protein coupled receptor, nematode specific	moderately reduced
F10A3.13	7-Helix G-protein coupled receptor, nematode specific	much reduced
F17A2.7	7-Helix G-protein coupled receptor, nematode specific	moderately reduced
F47C12.3	7-Helix G-protein coupled receptor, nematode specific	moderately reduced
F49C5.6	7-Helix G-protein coupled receptor, nematode specific	reduced
T07C12.1	7-Helix G-protein coupled receptor, nematode specific	reduced
T07C12.5	7-Helix G-protein coupled receptor, nematode specific	distorted
Y17G9A.d	7-Helix G-protein coupled receptor, nematode specific	distorted
Y94A7B.3	7-Helix G-protein coupled receptor, nematode specific	reduced
Y9C9A_53.c	7-Helix G-protein coupled receptor, nematode specific	reduced
T04A11.8	7TM receptor	reduced
F33G12.2	G-protein beta WD-40 repeats	Moderately reduced
<b>ION CHANNELS/PERMEASES/TRANSPORTERS (12)</b>		
C32C4.1	voltage-dependent potassium channel	distorted
B0310.1	potassium channel/very weak mammalian similarity	reduced
C37A5.1	homology Best's macular dystrophy (BMD) ion exchanger	slightly reduced
K04E7.2	PepT1 oligopeptide symporters	reduced
C34G6.4	ABC transporter	much reduced
K05F1.6	organic solute carrier family 2/ (OCT1)	moderately reduced

ZK682.2	sugar transporter	distorted
C13D9.7	sodium/calcium exchanger protein	reduced
F23F1.6	high affinity cationic amino acid permease	moderately reduced
F15H10.4	lysosomal amino acid transporter	reduced, distorted
F59F5.1	monocarboxylate transporter/XPCT	reduced
C46F11.1	unc-93 protein/ABC-2 type transporter	moderately reduced
<b>NEURONAL (4)</b>		
H27A22.1	glutaminyl cyclase/biosynthesis of pyroglutamyl peptides	distorted
T19B4.6	DCC/axon guidance/Fibronectin type III domain	reduced
T19B4.7	DCC/axon guidance/Fibronectin type III domain	much reduced
T27F7.1	neuroendocrine differentiation factor	much reduced
<b>ENERGY METABOLISM (6)</b>		
C33A12.1	NADH-ubiquinone oxidoreductase B subunit	reduced
F28H6.2	mitochondrial energy transfer proteins/carrier protein	distorted/reduced
F20D1.9	mitochondrial carrier proteins/similarity to uncoupling protein	moderately reduced
F14D12.2	cytochrome c family heme-binding site	moderately reduced
K12B6.8	cytochrome c family heme-binding site	distorted
C15H9.7	kynureninase	reduced
<b>VESICULAR TRANSPORT (11)</b>		
W03C9.3	RAB7	moderately reduced
F11A5.3	similarity to RAB2	moderately reduced
R11A5.1	beta-nap protein like/Adaptin	much reduced
F53H8.1	clathrin adaptor medium chain	reduced
T14D7.3	Synaptobrevin	slightly reduced
R01H2.3	sortilin (LDL receptor) family	reduced
T22D1.4	glycotransferase/ribophorin I	reduced
F54H5.3	VAMP -associated protein	reduced
C05E11.2	vacuolar protein sorting, vps16 like	distorted
K09B11.9	uso/p115 homology	moderately distorted
Y38E10A.c	similar to RIM binding protein 1A (rab-3 interacting protein)	moderately distorted
<b>PROTEIN DEGRADATION (5)</b>		
C49C3.3	ubiquitin family	moderately reduced
F49E12.4	ubiquitin-conjugating enzymes	moderately reduced
F52C6.2	ubiquitin domain	reduced
Y65B4B_10.a	putative ubiquitin-protein ligase	slightly reduced
Y65B4B_10.e	putative ubiquitin-protein ligase	moderately reduced
<b>CELL SURFACE/STRUCTURAL (8)</b>		
F40H3.5	heparan sulfate sulfotransferase	reduced
ZK39.7	chondroitin sulfate proteoglycan	much reduced
F49E11.4	extracellular proteins SCP/Tpx-1/Ag5/PR-1/Sc7 homology	distorted
K02D7.3	collagen triple helix repeat	much reduced
M01E10.2	collagen type XIV	reduced
Y77E11A_344i	collagen	reduced
F07A5.1	innexin	moderately reduced
F26D11.10	innexin	reduced
<b>CYTOSKELETAL (7)</b>		
Y37D8A.1	actin related protein 2/3 complex	distorted
Y17G7B.15	centaurin beta5	reduced

R107.6	CLIP-associating protein 1/microtubule dynamics	distorted
C23F12.1	endothelial actin-binding protein repeats	reduced
M106.5	F-actin capping protein beta subunit	distorted
C06G3.2	kinesin motor domain	reduced
T28D6.2	tubulin family protein	reduced
<b>GENERAL CELLULAR MACHINERY (11)</b>		
W09D10.3	mitochondrial ribosomal protein L1	reduced
W10D9.5	mitochondrial transport/Tom2 homology	reduced
W09D10.3	mitochondrial ribosomal protein L1	reduced
F54D5.11	TFIIE beta subunit core domain	reduced
F44B9.7	replication factor C-subunit	reduced
Y71H10B.1	5'-nucleotidase (purine), cytosolic type B	reduced
F21D5.5	polynucleotide kinase 3' phosphatase	distorted
F20D12.2	germinal center associated nuclear protein/DNA primase	much reduced
Y41D4A_3073.a	nucleoporin 155	much reduced
T22D1.10	rvvB-like DNA helicase	reduced
C24A1.4	transposase	much reduced
Y37A1A.1	set domain and mariner transposase fusion gene	reduced
<b>NO FUNCTION ASSIGNED (140)</b>		
W09G3.4	thiamine pyrophosphate enzyme/RCC1 and WD-40 repeat	distorted
H25K10.1	similarity to ser/thr protein phosphatase	distorted
C30F12.1	strong similarity to a hypothetical protein KIAA1726	much reduced
W09G3.1	similar to IAA0329 gene product	distorted
Y51H4A.m	hypothetical protein HDCMC04P	moderately reduced
Y48C3A.b	may be in transposase Tc1/Tc3 family	much reduced
F13E6.1	similarity to (NM_025741)	reduced
T21D12.3	polyglutamine binding protein 1/scurfy 2 candidate	reduced
B0041.5	TonB-dependent receptor protein/solute carrier protein	slightly reduced
B0286.4	similarity to AF113226 expressed in human heart tissue	reduced
D1054.14	hypothetical protein XP_061203	much reduced
F54C9.9	similarity to hypothetical protein FLJ12949	reduced
B0513.7	human homolog AF054989/may be transposase like	much reduced
Y41D4A_3192.a	strong similarity to human CAB66614	slightly reduced
ZK686.3	putative prostate cancer tumor suppressor	moderately reduced
B0041.3	putative peptidoglycan binding domain containing protein	distorted
C05E11.1	similarity to human homolog KIAA1715 protein	reduced
F38A5.1	strong similarity to a hypothetical protein FLJ11200	moderately reduced
C07E3.2	similar to DKFZP564C186 protein	moderately reduced
Y47G6A_245.b	similarity to hypothetical protein DKFZp434G1619.1	distorted
R05F9.8	similarity to S-crystallin/glutathione S-transferase	moderately reduced
W04A4.5	similar to CG12113	much reduced
Y49F6B.n	Red protein (RER protein/arginine (R) and glutamic acid (E)	distorted
F14D2.4	BTB/POZ/MATH (meprin and TRAF homology) domain	distorted
C30G4.5	D. melanogaster finger protein neuralized	moderately reduced
F58H1.6	EGF-like domain	much reduced
F55B11.4	Zinc finger, C2H2 type domain	slightly distorted
C47D12.7	BTB/POZ domain/Kelch repeat/human kelch 3 like	reduced
ZK355.d	domain of unknown function DUF41	reduced

C16C4.2	MATH (meprin and TRAF homology) BTB/POZ domain	moderately reduced
Y50D7_165.b	may be involved in mitochondria	reduced, thin
C54H2.5	surf4 family	reduced
T05F1.6	BRCT domain	slightly reduced
R08F11.2	Domain of unknown function DUF32	distorted
W01B11.5	proline-rich region	reduced
R05H11.1	F-box domain	reduced
C32D5.11	RING finger	moderately reduced
H32C10.3	DHHC-type Zn-finger Ankyrin-repeat/huntingtin interacting	much reduced
T21C9.2	Type-1 copper (blue) domain/VPS54	reduced
Y41E3.11	SPRY domain Proline rich extensin	reduced
T10D4.1	Domain of unknown function DUF19	slightly distorted
C54G7.1	weak homology to Vitamin K-dependent/(GLA) domain	some reduction
Y57G11C.17	weak homology to glycerol uptake protein	slightly reduced
T04C10.2	Yeast hypothetical protein L8167.6 like/epsin 2	moderately reduced
W06G6.1	weak similarity to RanBP7/importin	reduced
K12D12.4	weak similarity to zinc finger domain	distorted
C23H3.2	weak similarity to ATP-binding cassette protein ABCB9	much reduced
C56E10.3	weak similarity to desmoplakin	moderately reduced
T27E4.6	very weak similarity to FMLP-RELATED RECEPTOR II	moderately distorted
Y57A10A.bb	very weak similarity to XM_092364	reduced
C14A6.6	very weak similarity to transcription factor	reduced
Y67D8A_380.d	very weak similarity to AB052150)	slightly reduced
F08G2.7	very weakly similar to RCC domain	moderately reduced
F29B9.11	very weak similarity to alpha 1a-adrenoceptor	reduced
T26E4.13	very weak similarity to XM_089955)	reduced
H04M03.4	very weak similarity to lens fiber cell beaded-filament protein	much reduced
K02E7.11	very weak similarity to hypothetical protein AK057380	much reduced
T10C6.10	very weak similarity to a cytochrome P450	moderately reduced
Y57E12_242.c	very weak similarity to superkiller viralicidic activity	much reduced
T10E9.6	very weak similarity to mouse (BC020184)	moderately distorted
Y111B2C.e	very weak similarity to RIM2-4C	much reduced
Y71H2_389.a	very weak similarity to spondyloepiphyseal dysplasia	reduced
R160.4	very weak similarity to TNF	reduced
Y50E8.q	very weak similarity to anti-DNA immunoglobulin heavy chain	distorted, dumpy
F13B6.1	very weak similarity to vitamin D receptor	reduced
T21C9.11	very weak similarity to hypothetical protein XP_089285	distorted
T02H6.7	very weak similarity to arachidonate 5-lipoxygenas	reduced
F53A9.4	very weak similarity to hypothetical protein	reduced
C14F5.3	very weak similarity to troponin T	slightly reduced
C29H12.6	very weak similarity to an unknown protein	reduced, distorted
C56E6.4	very weak similarity to hypothetical protein XP_062076	reduced
F46C8.7	very weak similarity to an plasma membrane urea transporter	reduced
F46F5.10	very weak similarity to an rotocadherin 18 precursor	moderately reduced
C42C1.6	very weak similarity to hypothetical protein XM_087750	much reduced
F27C1.4	very weak similarity to hypothetical protein AK057039	reduced
B0554.7	very weak similarity to mouse NP_573485.1	moderately reduced
C18E9.5	very weak similarity to TRRAP protein	reduced

F28H7.6	very weak similarity to calcium-independent phospholipase	distorted
C08G5.2	very weak similarity to mouse membrane glycoprotein	distorted, dumpy
F54F7.2	very weak similarity to Complement C3 precursor	slightly reduced
M01A8.1	very weak similarity to Eph receptor	distorted, reduced
T01D3.4	very weak similarity to Rhodopsin-like GPCR superfamily	reduced
F59E11.5	very weak similarity to polyadenylation specificity factor 1	reduced
T19D7.1	very weak similarity to a serotonin receptor	reduced
B0554.6	very weak similarity to mouse ETL1,	reduced
ZC84.5	very weak similarity to thyroid receptor interacting protein 4	reduced
W05E10.2	very weak similarity to expressed sequence R74613	moderately distorted
ZK652.2	very weak similarity to (AJ011007)	moderately reduced
D2062.10	very weak similarity to adaptor-related protein complex AP-4	distorted
C17G10.7	very weak similarity to novel SH2-containing protein	moderately reduced
B0207.9	very weak similarity to hypothetical protein KIAA0153	reduced
F38E9.4	very weak similarity to cytoplasmic linker 2	distorted
Y65B4B_13.b	very weak similarity to ubiquitin protein ligase	reduced, hazy
Y38E10A.b	very weak similarity to a synaptotagmin 8	reduced
ZK593.3	very weak similarity to Laminin alpha-1 chain precursor	reduced
C14C6.8	very weak similarity to XP_040205.2	moderately reduced
F21H12.3	very weak similarity to chaperonin containing TCP1	slightly reduced
T23E1.1	very weak similarity to BC002298)	reduced
T17H7.1	very weak similarity to Ig heavy chain V	much reduced
F10A3.11	very weak similarity to vascular Rab-GAP/TBC-containing	moderately reduced
F41C6.6	very weak similarity to ABC family (CFTR/MRP)	reduced
T07C12.11	very weak similarity to synuclein alpha interacting protein	distorted
T14A8.1	very weak similarity to novel protein dj180E22.1	distorted
F58F9.1	very weak similarity to an apolipoprotein precursor	slightly reduced
ZK154.4	very weak similarity to chromodomain helicase	moderately distorted
R03H10.4	very weak similarity to MAPKKK 10	reduced
F55C12.3	very weak similarity to activin A type IB receptor precursor	moderately reduced
Y119D3_456.a	very weak similarity to guanylate binding protein (mouse)	reduced
F08D12.4	no significant mammalian homology	reduced
R11H6.6	no significant mammalian homology	reduced
Y57A10A.1	no significant mammalian homology	distorted
C14A4.12	no significant mammalian homology	distorted
Y7A9C.3	no significant mammalian homology	much distorted
C01G6.9	no significant mammalian homology	reduced
Y51H7C_255.c	no significant mammalian homology	reduced
B0041.5	no significant mammalian homology	slightly reduced
C15C7.5	no significant mammalian homology	reduced
T10C6.4	no significant mammalian homology	moderately reduced
Y37D8A.8	no significant mammalian homology	reduced
C50E10.5	no significant mammalian homology	reduced
ZK1290.1	no significant mammalian homology	reduced
F22E5.1	no significant mammalian homology	reduced
F12A10.8	no significant mammalian homology	reduced
B0034.2	no significant mammalian homology	reduced
F36H12.15	no significant mammalian homology	reduced

F52C6.12	no significant mammalian homology	much reduced
Y24D9A.b	no significant mammalian homology	distorted
K06B4.3	no significant mammalian homology	much reduced
T11F9.10	no significant mammalian homology	reduced
T19D2.3	no significant mammalian homology	reduced
T27E4.7	no significant mammalian homology	moderately distorted
Y105E8B.a	no significant mammalian homology	slightly reduced
Y69A2A_7278.	no significant mammalian homology	reduced, distorted
Y51H7B_5.b	no significant mammalian homology	slightly reduced
T13F2.6	no significant mammalian homology	distorted
K09H11.2	no significant mammalian homology	reduced
T26E4.10	no significant mammalian homology	reduced
T06H11.2	no significant mammalian homology	moderately reduced
F59F5.2	no significant mammalian homology	much reduced
ZK131.8	no significant mammalian homology	much reduced

### RNAi Clones that Reduce Fat and Viability/Growth

An additional 225 RNAi clones were identified that dramatically slowed development, or resulted in embryonic lethality. These genes are listed in Table VI.

5 Some of these RNAi clones interfered with the expression of previously identified genes with critical roles in fat biosynthesis and metabolism such as nematode acetyl-CoA carboxylase (W09B6.1), fatty acid synthase (F32H2.5), and fatty acid desaturase *fat-7*. The identification of these *C. elegans* homologs of mammalian genes also provides compelling evidence of the usefulness of *C. elegans* in identifying mammalian fat metabolism regulator genes critical to fat  
10 metabolism. The effects of other RNAi clones on development were attributed to the inactivation of genes that function in cellular maintenance (e.g., ATP synthesis, ribosomal biogenesis). This list identifies the target genes by *C. elegans* cosmid name and open reading frame number.

15 **Table VI. RNAi Clones that Reduce Fat Content and Reduce Viability/Growth**

<i>C. elegans</i> Gene	BRIEF DESCRIPTION	Nile Red Phenotype
F10D2.9	stearoyl-CoA desaturase	not fully grown, reduced
F29D11.1	low density lipid receptor-related protein	much distorted
F32H2.5	fatty acid synthase	much reduced, not grown
W06D12.3	fatty acid desaturase, type 1/stearoyl-CoA desaturase	reduced
w09b6.1	acetylCoA carboxylase alpha	much reduced, not grown
C09H10.3	Respiratory-chain NADH dehydrogenase 51 Kd subunit	much reduced
C53B7.4	ATP synthase	reduced, no progeny

F02E8.1	ATP synthase B chain	reduced
F35G12.10	ATP synthase B chain	much reduced
F37E3.1	cytochrome b6/CAP BINDING PROTEIN	distorted, reduced/no progeny
W04A8.7	taf-1/Cytochrome c family heme-binding site/Bromodomain	very hazy
Y110A7A.h	ATP synthase alpha and beta subunit	reduced
Y37D8A.14	cytochrome c oxidase subunit Va	much reduced
Y57G11C.12	NADH-ubiquinone oxidoreductase	reduced/no progeny
F28B3.1	cysteine proteases inhibitor	moderately
F59B2.12	eukaryotic thiol (cysteine) proteases active sites	reduced/not grown
C23H3.4	serine palmitoyltransferase	slow growth, reduced
C42C1.5	bacterial transferase hexapeptide repeat/ADP-glucose pyrophosphorylase	much reduced
D10I4.1	arylsulfatase E precursor	distorted
E04A4.7	cytochrome c, class IA and IB/Cytochrome C, Class I	hazy,, reduced
F01G10.1	Transketolase	reduced
F40H3.5	heparan sulfate sulfotransferase	moderately reduced
F44D12.4	LUT1 C-terminal binding protein/RGS-GAIP interacting protein GIPC	small, not grown, reduced
F46E10.1	AMP-dependent synthetase and ligase	reduced
F57B9.2	proline-rich region• Glycosyl hydrolases family 5	much reduced
H14A12.2	fumarate lyase	not grown, reduced
H15N14.2	AAA-protein (ATPases associated with various cellular activities) NSF	reduced
K02F2.2	S-adenosyl-L-homocysteine hydrolase	much reduced, no progeny
K06A4.5	3-hydroxyanthranilate 3,4-dioxygenase	distorted
T05H4.4	oxidoreductase FAD/NAD-binding domain	reduced
T05H4.5	oxidoreductase FAD/NAD-binding domain /cytochrome B5 reductase	much reduced
Y55F3A_750.e	weak similarity to putative~thioredoxin	much reduced, few progeny
B0285.1	serine/Threonine protein kinase family active site	much reduced/no progeny
C16C2.3	inositol-1,4,5-triphosphate 5-phosphatase	reduced
F10E9.7	RA domain/Proline-rich region/Pleckstrin homology (PH) domain	much reduced
W03F8.5	bacterial chemotaxis sensory transducer/EGF-domain/Laminin N-terminal	slight reduction/no progeny
W07E6.2	beta G-protein (transducin)/G-protein beta WD-40 repeats	reduced
ZK1067.1	let-23/tyrosine-protein kinase (Epidermal growth factor receptor subfamily)	much reduced
ZK675.1	HMGCR/Patched 5'TM bo3/PTC-2	few progeny, much reduced
C27B7.5	zn-finger CCHC type	reduced
C33D3.1	elt-2/zinc finger protein (GATA type)	much reduced
C34H3.a	C2H2-type zinc finger protein/odd-skipped-related 2A protein	reduced
D1081.2	MADS-box domain	distorted
F10C1.5	DM DNA binding domain	moderately
F22A3.1	prostate epithelium-specific Ets transcription factor	reduced/distortion
F23B12.7	EF-hand family/CCAAT BINDING FACTOR 1	distorted
F25H8.3	neutral zinc metallopeptidases/Thrombospondin type/reprolysin (M12B)	much distorted
W01D2.2	ligand-binding domain of nuclear hormone receptor	slightly reduced
		not fully grown, much reduced

Y17G7A.2	zinc finger, C2H2 type	lethal, not grown, reduced
C01F6.8	swelling-induced chloride conductance regulatory	much reduced
C56C10.8	TonB-dependent receptor protein/Nascent polypeptide Associated Complex	reduced, few progeny
C56E6.1	protein-dependent transport systems inner membrane component	reduced, no progeny
W06D12.2	potassium channel, subfamily K	not grown, reduced
W10D9.5	mitochondrial tranport/Tom2 homology	reduced,distorted
Y61A9LA_75.a	ABC transporters family	reduced
ZK105.e	sodium/potassium-transporting ATPase alpha-4 chain	reduced
C36B1.4	proteasome A-type subunit/Multispecific proteases of the proteasome	much reduced, no progeny
CD4.6	proteasome A-type subunit/Binding-protein-dependent transport systems	much distorted, reduced
F23F12.6	26S protease regulatory subunit.	much reduced
F39H11.5	multispecific proteases of the proteasome/YEAST NIP80 LIKE	much reduced, not grown
T23F2.1	glycosyl transferases group 1	reduced
Y38A8.2	proteasome B-type subunit/Multispecific proteases of the proteasome	not fully grown, much reduced
C36E8.5	beta tubulin	distorted
D2024.6	F-actin capping protein alpha subunit	reduced
F10C1.2	intermediate filament protein	reduced, no progeny
F20G4.3	myosin head (motor domain)/Myosin tail	distorted
F44F4.11	cell division protein FtsZ/tubulin	reduced
K07C5.1	actin-related protein 2; ARP2	reduced
T04C12.5	actin	much reduced
Y19D2B.1	alpha tubulin/FtsZ family	distorted, no growth
ZK593.5	CAP-Gly domain/dynactin	not fully grown, reduced
B0303.9	sec1 family/VPS33	reduced,
C02C6.1	dynamin 2	much reduced
C05D11.2	vps-16 like	not fully grown, much reduced
F29G9.3	clathrin adaptor comple3, small chain	much reduced
F41C3.4	got1 homology/vesicular transport	distorted no progeny
K02D10.5	synaptosomal associated protein	not fully grown, reduced
T21E12.4	dynein heavy chain	reduced, not much growth
ZK1014.1	NSF	not grown
B0222.6	nematode cuticle collagen N-terminal domain	moderately reduced
F57B9.5	similar to bystin-like	much reduced, no progeny
K01A6.4	Col IV similarity	reduced
K12D12.3	nematode collagen	much reduced, no progeny
W10C4.b	ankyrin-repeat	much reduced
B0035.7	histone H2A	much reduced/no progeny
B0035.8	histone H2B	not fully grown, reduced
B0035.9	histone 4 protein~putative	not fully grown,

B0041.4	ribosomal protein L4/L1e	reduced
B0495.6	unknown/weak similarity to eukaryotic translation initiation factor 3	much reduced, no progeny
C03C10.3	ribonucleotide reductase	reduced, /few progeny
C04H5.6	ATP-dependent helicase, DEAH-box	moderately reduced
C06A8.2	SNRNA ACTIVATING PROTEIN COMPLEX 43 KDA SUBUNIT	much reduced
C08B11.5	poly(A) RNA binding protein	no progeny, much reduced
C09H10.2	forkhead-associated (FHA) domain/ribosomal protein L36a-like	few progeny, much reduced
C15F1.e	translation initiation factor	reduced, not grown
C15H11.9	homolog of yeast ribosome biogenesis regulator	some distortion/few progeny
C16A3.3	18S and 5.8S rRNA synthesis	much reduced
C16A3.4	RNA-binding protein C2H2 Zn-finger domain/Zinc finger, C2H2 type	moderately reduced
C16A3.6	RNA binding protein	moderately reduced
C26D10.1	regulator of chromosome condensation (RCC1)/ran-1	not much growth, much distorted
C26F1.9	ribosomal protein L39e	much reduced
C27F2.4	putative methyltransferase	reduced
C29F5.3	cytidine and deoxycytidylate deaminase zinc-binding region	reduced
C37H5.8	heat shock protein hsp70	reduced, not grown
C42D4.8	DNA-directed RNA polymerase III largest subunit	reduced
C47D12.6	serine carboxypeptidase (S10)/Aminoacyl-transfer RNA synthetases	reduced, small droplets
C50F4.5	histone H2B	much reduced
C52A11.2	globin	reduced
C52E4.3	small nuclear ribonucleoprotein (Sm protein)	much reduced
D1007.6	40S ribosomal protein S10	reduced, not much growth
F09E8.3	DNA mismatch repair protein MutS family, C-terminal domain	moderately reduced
F09F7.3	RNA polymerases beta subunit	reduced
F18A1.5	replication protein A1 (70kD)	reduced
F20D12.4	centromere/kinetochor/laminin	no progeny
F22B3.1	histone H4	reduced
F22B5.2	translation initiation factor eIF3-p44	much reduced
F22B5.9	phenylalanyl-tRNA synthetase	reduced not grown
F22B5.9	phenylalanyl-tRNA synthetase beta-subunit	much reduced
F26F4.10	arginyl tRNA synthase/ligase	not grown, reduced
F26F4.11	DNA-DIRECTED RNA POLYMERASE	much reduced/no or few progeny
F32E10.4	serine-rich RNA polymerase I suppressor protein (SRP1)	much reduced/few progeny
F37C12.11	ribosomal protein S21e	much reduced
F37C12.9	ribosomal protein S11	not fully grown, much reduced
F45E12.3	cullin family/CELL CYCLE CONTROL	reduced
F45F2.13	histone H3	reduced, not grown
F54E12.1	histone H3	much reduced/no progeny

F54E12.5	histone H2A	not fully grown, reduced
F55C5.8	signal recognition particle 68 KD protein	much reduced, not grown
F55F10.1	no significant mammalian similarity	reduced
F55F10.2	sigma-54 factor interaction protein family	reduced/few progeny
F55G1.10	histone	much reduced, no progeny
F58A4.4	DNA primase small subunit	reduced, no progeny
H02I12.7	core histones H2A, H2B, H3 and H4	much reduced/no progeny
H06H21.3	eukaryotic initiation factor 1A	reduced/larger droplets/few progeny
H06I04.i	sbp homolog required for ribosomal biosynthesis	reduced
H19M22.1	cell migration/emb. Devel	Reduced
H23L24.c	N-6 Adenine-specific DNA methylase	much reduced/no progeny
K03A1.1	histone H3	Reduced
K05F1.5	hypothetical protein FLJ20321	moderately reduced/distortion
K12D12.2	nuclear pore comple3 homology	reduced, not fully grown
R05D11.3	nuclear transport factor 2 (NTF2) domain	much distorted/no progeny
R08D7.1	IDN3 homology/bud13	much reduced/no progeny
R11D1.8	ribosomal L28e protein family	moderately reduced
T01C3.6	ribosomal protein S9	not fully grown, reduced
T02G5.9	lysyl-tRNA synthetase	not fully grown
T03F7.5	aminoacyl-transfer RNA synthetases class-II	Distorted
T10C6.11	h• istone H2B	much reduced, no progeny
T10C6.12	histone H2A	much reduced
T10C6.13	histone H3	much reduced, no progeny
T13H5.4	RNA-binding protein C2H2 Zn-finger domain/PRP9 LIKE	much reduced
T23B12.2	ribosomal protein L4/L1e	Reduced
T28F3.2	heat shock protein hsp70	not fully grown, reduced
VW02B12L.1	V-type ATPase 116kDa subunit family	much reduced, dauer like
W07E6.1	NOL1/NOP2/sun family	reduced, droplet/no progeny
Y106G6H.3	ribosomal protein L30e	not grown, much reduced
Y41D4A_3073.a	nucleoporin 155	not fully grown, reduced
Y41D4A_3457.a	nup homology	not fully grown, reduced
Y41D4A_3457.d	nup homology	not fully grown, reduced
Y47D3A.c	DNA-directed DNA polymerase family B	reduced

Y62E10A.d	60S Acidic ribosomal protein	not fully grown, reduced
Y71G12A_187.bsnRNA-associated Sm-like protein		reduced
Y76B12C_66.c	cleavage and polyadenylation specific factor 1	reduced
ZK550.4	TFIIE alpha subunit	much reduced
ZK637.8	V-type ATPase 116kDa subunit family	much reduced, few progeny
ZK652.1	small nuclear ribonucleoprotein (Sm protein)	much reduced/no progeny
ZK686.1	ATP-dependent RNA helicase	much reduced
B0454.1	proline-rich region	reduced
B0491.5	very weak similarity to laminin	reduced
C02F12.8	proline-rich region	not fully grown, much reduced
C06A1.1	er94, VCP modulator of polyglutamine-induced neurodegeneration.	reduced
C10A4.4	unknown, very weak similarity to a hypothetical protein	moderately reduced, not grown
C14C10.3	no significant similarity	moderately reduced
C15H9.4	similarity Hypothetical protein KIAA1145	reduced
C16D9.5	unknown, very weak similarity to protein transport related protein	reduced
C18E9.4	very weak similarity to NADH-ubiquinone oxidoreductase	distorted/reduced
C29H12.6	no significant similarity	distorted, reduced, no progeny
C30B5.6	weak similarity to primitive neuroectodermal unknown protein/HSPC244	much reduced, no progeny
C30C11.2	domain in components of the proteasome, COP9-complex and eIF3 (PCI)	not fully grown, much reduced
C37H5.5	AD24 protein, UNNAMED PROTEIN PRODUCT	reduced, few progeny
C40D2.2	MATH (meprin and TRAF homology) domain	umpy, distorted
C42C1.3	very weak similarity to oxysterol binding protein	much reduced
C47C12.2	no significant mammalian similarity	not fully grown, distorted
D1054.3	suppressor of skp-1	much reduced
F08D12.7	no significant mammalian similarity	not fully grown, much reduced
F19F10.9	hypoxia associated factor	much distorted
F26A1.10	no significant mammalian similarity	slow growth, /much reduced
F29C4.2	no significant mammalian similarity	reduced, not grown
F32E10.1	similar to hypothetical protein FLJ14075	reduced/few progeny
F33A8.1	KIAA1604 protein	much reduced, not grown
F40H3.1	very weak similarity to an unknown protein	some distortion
F45C12.7	BTB/POZ domain	reduced
F45H10.4	no significant mammalian similarity	much reduced
F46C8.1	no significant mammalian similarity	much reduced
F47F6.4	very weak similarity to Langerhans cell specific c-type lectin	little growth, much reduced
F52C6.13	very weak similarity to steerin/solute carrier	much reduced
F54F2.7	similarity to unknown protein	reduced
F55C12.2	no significant mammalian similarity	reduced
F57G9.3	no significant mammalian similarity	reduced

F57G9.4	no significant mammalian similarity	reduced
H06I04.h	no significant mammalian similarity	distorted/reduced progeny
K02E7.6	very weak similarity to creatine kinase	reduced
K06A4.6	no significant mammalian similarity	reduced
K06A5.4	no significant mammalian similarity	distorted, much reduced/no progeny
R07E3.2	no significant mammalian similarity	slightly reduced
R12E2.2	membrane protein from human chromosome 1	no growth, reduced
R144.2	proline-rich region	distorted
T12A2.2	putative~related to OSTSTT3	moderately reduced
T19B10.2	no significant mammalian similarity	reduced
W01A8.4	no significant mammalian similarity	moderately reduced/no progeny
W01B11.5	proline-rich region	reduced
W02B3.7	no significant mammalian similarity	not fully grown, reduced
W04A4.6	no significant mammalian similarity	much reduced/no progeny
W07B3.2	no significant mammalian similarity	much reduced/few progeny
W10C6.1	repeat in APC and proteasome component	reduced
Y38F2A_5743.i	no significant mammalian similarity	much reduced
Y51H4A.m	weak similarity to hypothetical protein	moderately reduced
Y53C12B.2	similarity to gi 17390336 gb AAH18152.1 AAH18152	reduced
Y57A10A.v	no significant mammalian similarity	reduced
Y75B12B.3	no significant mammalian similarity	not grown/reduced
Y75B8A.27	very weak similarity to dynactin	not grown/reduced
ZK121.c	KIAA1002 protein; clone FLB5224	moderately reduced
ZK546.2	leucine-rich repeat	some distortion/no progeny
ZK795.3	domain of unknown function DUF96	reduced
C04G2.6	2Fe-2S Ferredoxin/Homeobox domain/Ribonuclease II domain	reduced/few progeny
F41H10.7	fatty acid elongase (CIG30/Fen1)	much reduced
T10B5.5	chaperonin subunit	mislocalized, slow growth

### RNAi Clones that Increase Fat Content

RNAi, followed by Nile Red staining, also identified genes whose inactivation caused an increase in fat content, but did not interfere with normal growth or development. Interestingly, increased fat content resulted from RNAi inactivation of the nematode homolog of a hepatocyte nuclear factor, *hnf-4α*. Mutations in human *HNF-4α* are associated with maturity onset diabetes of the young (Yamagata, et al., *Nature* 384:458-60, 1996). Increased fat phenotypes were observed when several cytochrome c P450 enzymes were inactivated. These enzymes may metabolize the ligands of nuclear hormone receptors affecting body fat.

Further evidence of common fat regulatory circuits in mammals and *C. elegans* came from the identification of several genes that appear to function similarly in regulating fat metabolism in nematodes and mammals. For instance, increased fat levels resulted from RNAi of C43H6.8, a potential ortholog of the hematopoietic/neurogenic transcription factor Nhlh-2/Nscl-2. Mice bearing a knock-out of this transcription factor display hypogonadism and obesity (Good, et al., *Nat. Genet.* 15:397-401, 1997).

Genes whose inactivation results in increased fat content are listed in Table VII. The increased fat phenotypes were categorized as (i) much increased (i.e., at least 2.5-fold the fat content of wild-type nematodes); (ii) increased (i.e., at least 2-fold the fat content of wild-type nematodes); (iii) slightly increased (i.e., at least 1.5-fold increased). Such genes might be useful targets for drug development. For example, drugs that increase the activity of these genes would be expected to decrease fat storage. Drugs that decrease the activity of these genes would be expected to increase fat levels, which might be useful in treating, for example, cachexia. A condition associated with cancer and chemotherapy. This list identifies the target genes by *C. elegans* cosmid name and open reading frame number.

**Table VII. RNAi Clones that Increase Fat Content**

<i>C. elegans</i> Gene	Brief Description	Nile Red Phenotype
C33A12.6	UDP-glucoronosyl and UDP-glucosyl transferase	moderately increased
E04F6.3	MaoC-like dehydrogenase-epimerase-[17 beta HSD]	moderately increased
E04F6.6	Orn/DAP/Arg-type decarboxylases	moderately increased
F15B9.5	serine protease	moderately increased
F28F8.2	long chain fatty acid CoA synthetase/ligase	moderately increased
F47B8.3	glutaredoxin 3 /thioredoxin	moderately increased
VF13D12L.1	myo-inositol-1-phosphate synthase	increased, dumpy
C37F5.1	elk-1	slightly increased
C43H6.8	nhlh2/nscl-2	moderately increased
C56C10.10	aryl hydrocarbon receptor (Leber congenital amaurosis)	increased
C56E10.4	C4-type steroid receptor zinc finger	enlarged droplet
F16B4.9	C4-type steroid receptor zinc finger	moderately increased
F33D4.1	nuclear hormone receptor/estrogen-type	slightly increased
H12C20.3	C4-type steroid receptor zinc finger	moderately increased
K10C3.6	hepatocyte nuclear factor 4 receptor	moderately increased
R11H6.5	interleukin enhancer binding factor 2	increased
C04G2.2	serine/threonine protein kinase/tau tubulin kinase	slightly increased
C09G5.8	Protein interacting with retinitis pigmentosa GTPase	increased
C18H9.7	RAPSN (associated with nicotinic acetylcholine receptor)	increased
C24F3.2	glucokinase-associated dual specificity phosphatase	slightly increased

F39B1.1	phosphoinositide 3-kinase	moderately increased
F46C5.6	Protein phosphatase PP2A subunit A	moderately increased
F56D5.9	BRCT /ankyrin-repeat/protein phosphatase domains	moderately increased
F56H11.6	casein kinase/tau-tubulin kinase	moderately increased
K08F8.1	ribosomal S6 kinase	increased
R10D12.10	casein kinase/tau-tubulin kinase	increased
T04B2.2	fms/fps protein kinase	slightly increased
T04C9.1	oligophrenin-1 (focal adhesion GTPase)	moderately increased
W03A5.4	guanylate kinase associated protein	increased
W08D2.1	wnt-1 family kinase	moderately increased
Y11D7A.9	FGF receptor activating protein	much distortion, some increase
ZC513.1	permeability increasing/phospholipid transfer protein	increased when starved
C43H6.9	glutamate receptor	moderately increased
F08H9.5	cubilin/endocytic receptor	moderately increased
F56B6.5	major hippocampal somatostatin receptor	increased
T19D12.8	nematode specific 7-TM receptor	increased
Y27F2A.g	chemoreceptor	increased
Y40H7A.1	nematode specific G-coupled protein receptor	moderately increased
Y46H3C_11.b	rhodopsin-like GPCR superfamily	enlarged droplet
F32B6.9	vitelliform macular dystrophy protein/bestrophin	slightly increased
ZC410.4	potassium channel	slightly increased
C18H9.5	sugar transporter	increased
F14E5.1	glucose transporter-3	enlarged droplet
F52H2.2	amino acid permease	moderately increased
C04G2.4	vesicle associated protein	moderately increased
F32B6.6	Vamp-associated protein	moderately increased
C15A11.3	procollagen proteinase enhancer	moderately increased
C34F6.3	collagen triple helix repeat	moderately increased
C53B4.5	collagen triple helix repeat	slightly increased, distorted
EGAP7.1	collagen triple helix repeat	increased
F46C8.6	cuticle collagen	moderately increased
T14B4.7	collagen triple helix repeat	increased, dumpy
T28C6.6	collagen triple helix repeat	moderately increased
Y38F1A.9	contactin 6/myopalladin	moderately increased
Y41E3.2	collagen triple helix repeat	increased, dumpy
K02D7.5	recombination activating gene	moderately increased
C04G2.5	very weakly similar to AK027463	slightly increased
C09G12.5	very weakly similar to CREB-binding protein	slightly increased
C14A4.1	strongly similar to CG2245 gene product [Mus musculus]	moderately increased
C24F3.1	no significant mammalian homology	slightly increased
C33A12.14	very weakly similar to fibronectin 2	moderately increased
C36A4.5	claustrine like/very weak similarity to bile acid activated lipase	moderately increased
C44E4.5	similarity to chronic myelogenous leukemia tumor antigen 66	moderately increased
C50C10.4	no significant mammalian homology	increased
C50D2.1	no significant mammalian homology	moderately increased
D1007.5	similar to hypothetical protein XM_147195	moderately increased
F12E12.h	very weakly similar to AB028991	increased

F25G6.9	very weakly similar to AK056522	moderately increased
F25H8.1	strong similarity to unknown protein AK056522	slightly increased
F25H8.2	very weakly similar to NM_138386	slightly increased
F25H8.5	proline-rich region	distorted
F26H9.4	strong similarity to unknown protein XM_135042	increased
F31F6.2	very weakly similar to XM_067663)	increase
F42G8.5	very weakly similar to BC030641	slightly increased
F44D12.7	Major sperm protein (MSP) domain	moderately increased
F49C12.15	no significant mammalian homology	moderately increased
F49F1.4	no significant mammalian homology	increased
F52C12.2	no significant mammalian homology	enlarged droplet
F56B3.2	no significant mammalian homology	moderately increased
F56F3.4	AN1-like Zinc finger/Ubiquitin domain	moderately increased
H05L03.3	no significant mammalian homology	increased
H05L14.2	very weakly similar to zinc finger protein NY-REN-4	moderately increased
K01G5.8a	very weakly similar to AB041658	increased
K02E10.3	no significant mammalian homology	increased
K02E10.5	very weakly similar to AE006464	moderately increased
K07A1.13	very weakly similar to cytochrome P450,	moderately increased
K07E8.3	very weak similarity to activin interactin protein	slightly increased
K09C4.5	very weak similarity to AraC HIH/Sugar transporter domain	increased
LLC1.2	very weakly similar to hypothetical protein XP_095577	slightly increased
M70.1	Domain of unknown function (WSN)	slightly increased
M70.3	no significant mammalian homology	increased
R07A4.2	very weakly similar to nectin-like protein 1	increased
R105.1	very weakly similar to KIAA1048 protein	some increase
T01C1.2	very weakly similar to NM_053797) crooked neck protein	slightly increased
T02C5.3	very weakly similar to neural cell adhesion molecule	moderately increased
T04C9.2	no significant mammalian homology	moderately increased
T05E8.2	very weakly similar to germ cell-specific gene 2	increased
T07F8.1	very weakly similar to neurofilament, medium polypeptide	slightly increased
T12A2.5	no significant mammalian homology	moderately increased
T12B5.8	F-box domain/Domain of unknown function DUF38	slightly increased
T14B1.1	very weakly similar to plexin 3	increase
T14B4.8	no significant mammalian homology	moderately increased
T14F9.4	very weakly similar to BC001973	moderately increased
T19D12.3	very weakly similar to polyadenylation specificity factor 3	slightly increased
T27A8.4	very weakly similar to NA repair protein XRCC1	moderately increased
W06H12.1	similar to hypothetical protein MGC4054	increased
Y11D7A.8	very weakly similar to XM_163806	moderately increased
Y47D9A.e	no significant mammalian homology	Increased
Y57A10B.1	similar to hypothetical protein AK005032	slightly increased
Y5H2B.e	very weak similarity to G protein-coupled receptor GPR26	moderately increased
Y67A6A.1	no significant mammalian homology	increased
Y73C8C.4	very weakly similar to AB055252	moderately increased
ZC64.2	transthyretin-like family	slightly increased
ZK1320.10	very weakly similar to XM_164500	moderately increased

ZK1321.1	very weakly similar to slit homolog	slightly increased
ZK666.10	very weakly similar to XM_088171	moderately increased

### Epistasis Analysis Orders Genes in Fat Metabolism Regulatory Pathway

RNAi was also used to inactivate genes in mutant genetic backgrounds. For this epistasis analysis, mutant nematodes with increased fat phenotypes including *lpo-1*, *daf-2(e1370)*, *tub-1(nr2004)*, and *tph-1(mg280)* were grown on RNAi bacteria, which had been shown to cause a reduced fat phenotype in wild-type nematodes, stained with Nile Red, and examined. In most cases, the RNAi clone caused a reduced fat phenotype in the increased fat mutant background. This indicated that the reduced fat RNAi gene was epistatic to the increased fat mutant gene. In some cases, however, the fat content of the increased fat mutant nematodes was unaffected by a reduced fat RNAi clone. Analysing such epistatic relationships among fat metabolism regulator genes allows the genes to be ordered in a pathway. The results of this epistasis analysis are shown in Tables VIIIA and VIIIB. Those RNAi clones that failed to produce a change in fat content are denoted by an **F**. Those RNAi clones that reduced fat content or altered fat droplet morphology are denoted with an **O**. Those RNAi clones that were not tested in a particular genetic background are denoted with an ND (Not Done).

**Table VIIIA. Epistasis Analysis on RNAi Targets that Reduce Fat without Reducing Viability**

Wild-type	<i>tph-1(mg280)</i>	<i>tub-1(nr2004)</i>	<i>daf-2(e1370)</i>	<i>lpo-1</i>	<i>lpo-6</i>	
O	O	O	O	O	O	C34G6.4
O	O	O	O	O	O	ZK675.1
O	O	O	O	O	O	F11E6.5
O	O	O	O	O	O	K07C6.5
O	O	O	O	O	O	F59F5.2
O	O	O	O	O	O	F13D11.1
O	O	O	O	O	O	T14E8.3
O	O	O	O	O	O	Y47D3B.7
O	O	O	O	O	O	C30F12.1
O	O	O	O	O	O	F52C6.12
O	O	O	O	O	O	Y119D3_456.a
O	O	O	O	O	O	ZK131.8
O	O	O	O	O	F	K09D9.2
O	O	O	O	O	O	W10D9.5
O	O	O	O	O	O	F53H8.1

O	O	O	O	O	O	C49C3.3
O	O	O	O	O	O	Y41D4A_3073.a
O	O	O	O	O	O	Y71H10B.1
O	O	O	O	O	O	C15C7.5
O	O	O	O	O	F	K04E7.2
O	O	O	O	O	O	F54D5.11
O	O	F	O	O	O	F46G11.3
O	O	O	O	O	O	F15H10.4
O	F	F	O	O	F	Y57A10A.bb
O	F	F	F	O	F	F41H10.7
O	O	O	O	O	F	F13D12.6
O	O	O	F	O	O	F20H11.2
O	F	O	O	O	O	T01D3.4
O	O	O	F	O	O	F52C6.2
O	O	O	F	O	O	Y37D8A.1
O	F	F	F	F	O	Y50E8.q
O	O	O	O	O	O	C06E7.3
O	O	O	O	O	O	K10B3.7
O	O	O	F	O	O	F49E11.4
O	O	F	O	O	O	F11A10.3
O	O	O	O	O	O	F38E9.4
O	O	O	O	F	O	K09H11.2
O	O	O	O	F	O	T04C10.2
O	F	O	O	O	O	Y51H4A.m
F	O	O	O	O	F	M01B12.5
O	O	O	F	O	O	F47C12.3
O	F	O	O	O	O	F22A3.5
O	O	F	F	O	O	R11A5.1
O	O	F	O	O	O	F49E12.4
O	O	F	O	O	F	C06G3.2
O	O	O	F	O	O	T28D6.2
O	O	O	F	O	O	D2089.2
O	O	O	F	O	O	C32D5.11
O	F	F	O	O	O	H04M03.4
O	F	O	F	O	O	Y57E12_242.c
O	O	O	F	O	F	F37B12.3
O	F	O	O	O	O	C33A12.1
O	F	O	O	O	O	C13D9.7
O	O	O	O	O	F	K02D7.3
O	O	F	O	O	O	R04A9.4
O	O	O	F	O	F	F20D12.2
O	F	F	O	O	O	W09G3.1
O	O	O	F	O	O	Y111B2C.e
O	O	F	O	O	F	T19D2.2
O	O	O	O	F	F	F07C4.1
O	O	O	O	F	F	T07C12.5
O	F	F	O	O	F	K08A2.b

O	O	F	F	F	O	Y65B4B_10.e
O	O	O	F	O	F	M106.5
O	O	O	F	F	O	W09D10.3
O	F	F	F	O	F	Y48C3A.b
O	O	F	O	O	F	B0554.6
O	F	O	F	O	O	C07E3.2
O	O	F	O	O	F	C47D12.7
O	F	F	O	O	O	F10A3.11
O	F	F	O	O	O	F29B9.11
O	O	O	O	O	F	T19D2.3
O	O	O	O	O	O	ZC84.5
O	O	F	O	O	O	R07B7.9
O	F	F	O	O	O	F52B11.2
O	F	O	F	O	F	F28H6.2
O	F	F	O	O	O	F10A3.13
O	F	F	O	O	O	Y77E11A_3443.i
O	O	O	F	O	F	F44B9.7
O	O	F	F	O	O	T22D1.10
O	O	F	O	O	F	F38A5.1
O	F	F	O	O	O	T21D12.3
O	F	O	O	O	F	W05E10.2
O	O	F	F	O	F	Y54G9A.2
O	O	O	O	F	F	ZK593.3
O	F	F	F	O	O	C34C6.6
O	O	O	ND	O	F	B0218.5
O	O	F	F	O	O	C07A9.2
O	O	O	F	O	F	E02C12.3
O	F	F	F	O	F	H09F14.1
O	O	F	F	F	O	T07C12.1
O	F	F	O	O	F	B0280.3
O	F	F	F	O	O	C01G6.5
O	F	F	F	O	O	Y116A8C.32
O	F	F	O	O	F	ZK686.4
O	F	O	F	O	F	T19B4.7
O	F	F	F	O	O	T27F7.1
O	O	F	F	O	F	F26D11.10
O	F	O	O	O	F	C18E9.5
O	F	F	F	O	O	D1054.14
O	O	F	O	O	F	F28H7.6
O	O	O	F	F	F	K06B4.3
O	F	F	O	O	O	W04A4.5
O	F	F	F	O	O	Y7A9C.3
O	O	O	F	O	F	ZK652.2
O	O	F	F	O	F	AH10.1
O	O	O	F	F	F	F11C1.6
O	O	F	O	O	F	Y49A3A.1
O	O	F	F	O	F	C24A11.9

O	O	F	F	O	F	F43H9.2
O	F	F	F	O	O	Y55F3C.c
F	O	F	F	O	F	K12B6.8
O	O	F	F	O	O	C47D12.1
O	F	O	O	O	F	F41D9.1
F	F	F	O	O	O	T04D3.2
O	F	F	F	O	O	Y44A6B.2
O	F	F	F	O	O	Y9C9A_53.c
O	F	O	O	F	F	C32C4.1
O	F	F	O	O	F	F11A1.3
O	F	F	O	O	F	T09F3.1
O	F	F	O	F	F	H27A22.1
O	F	F	F	O	O	C05E11.1
O	F	F	O	O	F	C14A6.6
O	F	F	F	O	O	C42C1.6
O	O	O	F	O	F	F13B6.1
O	F	F	F	O	O	H32C10.3
O	F	F	O	F	F	K12D12.4
O	F	F	O	O	F	R08F11.2
O	O	O	F	F	F	R11H6.6
O	F	F	F	O	O	Y37D8A.8
O	O	O	O	O	O	Y40H7A.7
O	F	O	F	O	O	Y41D4A_3192.a
O	O	F	O	O	O	Y65B4B_13.b
O	F	F	F	O	O	C37H5.3
O	F	O	F	F	F	C54G7.1
O	F	F	O	O	O	C33H5.17
O	F	F	F	O	O	F45H7.4
O	O	F	F	F	F	W03C9.3
O	O	F	F	O	F	Y53C12A.1
O	F	F	O	O	F	ZC302.1
O	O	F	F	F	O	F33G12.2
O	O	F	O	O	F	K05F1.6
O	O	F	F	F	O	F22A3.4
O	F	F	F	O	F	W02C12.3
O	F	F	F	O	F	ZK39.7
O	F	F	F	O	O	ZK757.3
O	F	F	O	O	F	B0034.2
O	F	F	O	O	F	B0041.3
O	O	F	F	O	F	B0286.4
O	F	F	O	O	F	B0554.7
O	O	F	O	O	F	C30G4.5
O	O	F	F	F	O	C56E6.4
O	F	O	F	F	F	F41C6.6
O	F	F	O	F	O	K02E7.11
O	F	F	O	F	F	R05H11.1
O	F	F	O	O	F	T10D4.1

O	F	F	O	F	O	T26E4.13
O	F	F	O	F	O	Y105E8B.a
O	F	F	O	F	O	Y24D9A.b
O	F	F	F	F	O	Y41E3.10
O	F	F	F	O	F	T12A2.1
F	O	ND	F	F	F	C02F4.2
O	O	F	F	O	F	C16A11.3
O	F	F	F	F	O	Y69A2A_7278.1
O	O	F	F	O	F	C06G3.10
O	F	F	O	O	F	F11A5.3
O	F	F	F	O	F	T22D1.4
O	F	F	F	O	F	R107.6
O	F	F	F	O	F	Y17G7B.15
O	F	F	F	O	F	C23H3.2
O	F	F	F	O	F	F12A10.8
O	F	F	F	O	F	F14D2.4
F	F	F	O	O	F	F46F5.10
O	F	F	F	F	O	F58H1.6
O	F	F	O	F	F	T17H7.1
O	F	O	O	O	O	Y51H7B_5.b
F	O	F	O	F	F	R13H8.1
F	O	F	O	F	F	T07A9.6
O	F	F	F	O	F	F14H8.1
O	F	F	F	O	F	F15A8.6
O	F	F	F	O	F	E01A2.i
O	F	F	F	O	F	H04M03.1
O	ND	ND	F	ND	F	C15H9.7
O	F	F	F	O	O	C06A1.3
F	F	F	F	O	F	C41D7.2
O	F	F	F	O	F	H25K10.1
O	ND	F	F	O	F	R107.4
O	F	F	O	F	F	ZC504.4
O	F	F	F	O	F	F58G4.2
O	F	F	F	O	F	T04A11.8
O	F	F	F	O	F	F59F5.1
O	F	F	O	F	F	ZK682.2
O	F	O	F	F	F	C33G8.9
O	F	F	F	F	O	F55B11.4
O	F	F	F	O	F	Y4C6A.h
O	F	F	F	O	F	F40H3.5
O	F	F	F	O	F	C05E11.2
O	F	F	F	O	F	M01E10.2
O	F	F	F	O	F	C23F12.1
O	ND	ND	ND	O	ND	B0207.9
O	F	F	F	O	F	B0513.7
O	O	F	F	F	F	C01G6.9
O	F	F	F	F	O	C08G5.2

O	ND	ND	ND	O	ND	C29H12.6
O	F	F	F	O	O	C50E10.5
O	F	F	F	O	O	F21H12.3
O	F	F	F	O	F	F27C1.4
F	F	F	F	O	F	F36H12.15
O	F	F	F	O	F	F39D8.2
O	ND	ND	ND	O	ND	F54C9.9
O	O	F	F	F	F	R03H10.4
O	F	F	F	O	F	T02H6.7
O	F	F	F	O	F	T07C12.11
F	F	F	O	O	F	T14A8.1
O	F	F	O	F	F	T21C9.11
O	F	F	O	O	F	T21C9.2
O	F	F	F	F	O	T23E1.1
O	F	F	F	O	O	W06G6.1
O	F	F	F	O	F	Y38E10A.b
O	O	O	O	O	O	Y47G6A_245.b
O	F	O	F	O	F	Y51H7C_255.c
O	F	F	F	O	F	Y69A2A_7278.m
O	F	F	F	F	O	Y71H2_389.a
O	O	F	F	F	F	ZK1290.1
O	F	F	F	O	F	ZK355.d
O	F	F	F	O	F	C17C3.1
O	F	F	F	O	F	ZK6.7
O	F	F	F	F	F	F28H6.3
O	F	F	O	O	F	Y6B3B.10
O	F	F	F	O	F	T04A8.16
O	F	O	O	O	O	F14D12.2
O	F	F	F	O	F	F07C3.4
O	F	F	F	O	F	T05C12.1
O	F	F	O	F	F	C38C10.1
O	F	F	O	O	F	F17A2.7
O	F	O	O	O	F	C37A5.1
O	F	F	F	O	F	F54H5.3
O	F	F	ND	O	F	K09B11.9
O	F	F	F	O	F	Y65B4B_10.a
O	F	F	F	O	F	F07A5.1
O	F	F	F	O	F	Y37A1A.1
O	F	F	F	O	F	C14F5.3
O	F	F	F	O	F	F13E6.1
O	F	F	F	O	F	F46C8.7
O	F	F	F	O	F	F55C12.3
O	F	F	F	F	O	F59E11.5
O	F	F	F	O	F	M01A8.1
O	F	F	O	O	F	R160.4
O	F	F	O	O	F	T10E9.6
O	F	F	O	O	F	T19D7.1

O	F	F	F	O	F	T27E4.7
O	F	F	F	O	F	Y41E3.11
O	F	F	F	O	F	Y49F6B.n
O	F	F	F	O	F	C01C10.3
O	F	F	F	O	F	F23H11.9
O	O	F	F	O	O	Y57G11C.17
O	F	F	F	O	F	K07C6.4
O	F	F	F	F	F	T08B2.7
O	F	F	F	F	F	F20D1.9
O	F	F	F	F	F	C44F1.5
O	F	F	F	O	F	K10D3.5
O	F	F	F	O	F	R07E5.1
O	F	F	F	ND	F	ZK909.3
O	F	F	F	F	F	B0041.5
O	F	O	F	O	F	B0310.1
O	F	F	F	O	F	C46F11.1
O	F	F	F	O	F	F23F1.6
O	F	F	F	F	F	C47C12.3
O	F	F	F	O	F	T23F11.4
O	F	F	F	O	F	T14D7.3
O	F	F	F	F	F	C24A1.4
O	F	F	F	O	F	C14C6.8
O	O	O	O	O	O	C56E10.3
O	ND	ND	ND	O	ND	F08D12.4
O	ND	ND	ND	O	ND	F08G2.7
O	O	F	F	F	F	F54F7.2
O	F	F	F	F	O	T10C6.4
O	O	F	F	O	F	T27E4.6
O	F	F	F	O	F	Y50D7_165.b
O	F	F	F	O	O	Y67D8A_380.d
O	F	F	F	F	F	ZK686.3
F	O	F	F	F	F	F25E2.5
O	F	F	F	F	F	B0285.8
O	F	F	F	F	F	C15B12.7
O	F	F	F	F	F	C44E4.6
O	F	F	F	F	F	F31F6.7
O	F	F	F	F	F	K02D3.2
O	F	F	F	F	F	M05B5.4
O	F	F	F	O	F	W01C9.4
O	F	F	F	F	F	M28.6
O	F	F	F	F	F	T09B4.8
O	F	F	F	F	F	K03B8.3
O	F	F	F	F	F	C03D6.3
O	F	F	F	O	F	C06A6.1
O	F	F	F	F	F	F45E4.6
O	F	F	F	F	F	H08M01.2
O	F	F	F	F	F	W09G3.4

O	F	F	F	F	F	ZK930.1
O	F	F	F	F	F	F49C5.6
O	F	F	F	O	F	Y17G9A.d
O	F	F	F	F	F	Y94A7B.3
O	F	F	F	O	F	C09G9.7
O	F	F	O	F	F	T19B4.6
O	F	F	F	F	F	F21D5.5
O	F	F	F	F	F	C14A4.12
O	ND	ND	ND	F	ND	C54H2.5
O	F	F	F	F	F	D2062.10
O	F	F	F	F	F	F22E5.1
O	ND	ND	ND	F	ND	F53A9.4
O	F	F	F	O	F	F58F9.1
O	F	F	F	F	F	T10C6.10
O	F	F	F	F	F	T11F9.10
O	F	F	F	F	F	T26E4.10
O	ND	ND	ND	ND	ND	W01B11.5
O	O	O	O	O	O	Y57A10A.1
O	F	O	F	F	F	ZK154.4
O	F	F	F	F	F	C46H11.2
O	F	F	F	F	F	R05F9.8
O	F	F	F	F	F	C31H2.3
O	F	F	F	F	F	C46E10.9
O	F	F	F	F	F	C16C4.2
O	F	F	O	O	F	T05F1.6
O	F	F	F	F	F	T06H11.2
O	F	F	F	F	F	Y38E10A.c
F	F	F	F	F	F	L4440

**Table VIIIB. Epistasis Analysis on Genes that Reduce Fat and Growth/Viability**

Wild type	<i>tph-1(mg280)</i>	<i>tub-1(nr2004)</i>	<i>daf-2(e1370)</i>	<i>lpo-1</i>	<i>lpo-6</i>	
O	o	o	o	o	o	F10D2.9
O	o	o	o	o	o	F29D11.1
O	o	o	o	o	o	F32H2.5
O	o	F	ND	F	o	W06D12.3
O	o	o	o	o	o	w09b6.1
O	o	o	o	o	F	C09H10.3
ND	ND	ND	ND	ND	ND	C53B7.4
O	o	o	o	o	F	F02E8.1
O	o	o	o	o	o	F35G12.10
O	o	o	o	o	o	F37E3.1
O	o	o	o	o	F	W04A8.7
O	o	o	o	o	F	Y110A7A.h
O	o	o	o	o	o	Y37D8A.14
O	o	o	o	o	o	Y57G11C.12
O	F	F	F	F	F	F28B3.1

O	F	F	o	o	o	F59B2.12
O	o	o	o	o	o	C23H3.4
O	F	o	F	o	o	C42C1.5
O	o	o	o	o	o	D10I4.1
O	o	o	o	o	o	E04A4.7
O	o	o	o	o	o	F01G10.1
O	o	o	F	o	o	F40H3.5
O	o	o	o	o	o	F44D12.4
O	o	o	o	o	o	F46E10.1
O	o	o	o	o	F	F57B9.2
O	o	F	o	o	F	H14A12.2
O	o	o	o	o	o	H15N14.2
o	o	o	o	o	o	K02F2.2
o	o	o	o	o	o	K06A4.5
o	o	F	o	o	F	T05H4.4
o	o	o	o	o	F	T05H4.5
o	o	o	o	o	o	Y55F3A_750.e
o	F	F	F	o	F	B0285.1
o	o	o	F	o	o	C16C2.3
o	o	o	o	o	o	F10E9.7
F	o	o	o	o	F	W03F8.5
o	F	F	o	o	F	W07E6.2
o	ND	ND	ND	ND	ND	ZK1067.1
o	o	o	o	o	o	ZK675.1
o	F	F	F	o	F	C27B7.5
o	F	o	o	o	ND	C33D3.1
o	o	o	o	o	o	C34H3.a
o	F	o	o	o	F	D1081.2
o	o	o	o	o	F	F10C1.5
o	o	o	o	o	o	F22A3.1
o	o	F	F	F	F	F23B12.7
F	o	o	o	o	F	F25H8.3
o	o	o	o	o	o	W01D2.2
o	o	o	o	o	o	Y17G7A.2
o	o	F	o	o	o	C01F6.8
o	o	o	F	o	F	C56C10.8
o	o	o	o	o	F	C56E6.1
o	F	F	F	F	o	W06D12.2
o	ND	ND	ND	o	ND	W10D9.5
o	o	o	o	o	F	Y61A9LA_75.a
o	o	o	o	o	F	ZK105.e
o	o	o	o	o	o	C36B1.4
o	o	o	o	o	o	CD4.6
o	o	o	o	F	o	F23F12.6
o	o	o	o	o		F39H11.5
o	o	o	o	ND	o	T23F2.1
o	o	o	o	o	o	Y38A8.2
o	o	o	o	o	o	C36E8.5

o	F	F	F	o	F	D2024.6
ND	ND	ND	ND	ND	ND	F10C1.2
o	F	F	F	o	F	F20G4.3
o	o	o	o	o	o	F44F4.11
o	o	o	o	o	o	K07C5.1
	o	o	o	F	o	T04C12.5
o	o	F	o	o	o	Y19D2B.1
o	F	o	F	o	F	ZK593.5
o	o	o	o	o	o	B0303.9
	o	o	o	v	o	C02C6.1
o	o	F	o	o	o	C05D11.2
o	o	o	o	o	o	F29G9.3
						F41C3.4
o	o	o	o	o	o	K02D10.5
o	o	o	o	o	F	T21E12.4
o	o	o	o	ND	o	ZK1014.1
o	F	F	o	F	o	B0222.6
o	o	o	F	o	o	F57B9.5
o	o	o	o	o	F	K01A6.4
o	F	o	o	o	o	K12D12.3
o	o	o	o	o	o	W10C4.b
o	o	o,	o	o	o	B0035.7
o	o	o	o	o	o	B0035.8
o	o	o	o	o	o	B0035.9
o	o	F	F	o	o	B0041.4
o	o	o	o	o	o	B0495.6
o	o	o	F	o	o	C03C10.3
o	o	o	o	o	o	C04H5.6
o	o	o	o	o	o	C06A8.2
o	o	o	o	o	o	C08B11.5
o	o	o	o	o	o	C09H10.2
o	o	F	o	o	F	C15F1.e
o	o	o	o	o	o	C15H11.9
o	o	o	F	o	o	C16A3.3
o	o	o	o	o	F	C16A3.4
o	F	o	o	F	F	C16A3.6
o	o	F	o	o	F	C26D10.1
o	o	o	o	o	o	C26F1.9
o	F	o	o	o	o	C27F2.4
o	F	o	F	o	F	C29F5.3
o	o	o	F	o	o	C37H5.8
o	o	o	o	o	o	C42D4.8
o	o	o	o	o	F	C47D12.6
o	o	o	o	o	o	C50F4.5
o	F	F	o	o	F	C52A11.2
o	ND	o	o	o	o	C52E4.3
o	o	o	o	o	o	D1007.6
o	o	F	o	F	F	F09E8.3

0	0	F	0	0	F	F09F7.3
ND	0	ND	0	0	F	F18A1.5
0	F	F	F	0	0	F20D12.4
0	0	0	F	0	0	F22B3.1
0	0	0	0	0	0	F22B5.2
0	0	0	0	0	F	F22B5.9
0	0	F	F	0	0	F26F4.10
0	0	0	0	0	0	F26F4.11
0	0	0	F	0	0	F32E10.4
0	0	0	0	0	0	F37C12.11
0	0	0	0	0	0	F37C12.9
0	F	0	F	0	0	F45E12.3
0	0	0	0	0	0	F45F2.13
0	0	0	0	0	0	F54E12.1
0	0	0	0	0	0	F54E12.5
0	ND	0	0	0	0	F55C5.8
0	0	0	0	0	F	F55F10.1
0	0	0	0	0	F	F55F10.2
0	0	0	0	0	0	F55G1.10
0	0	0	F	0	0	F58A4.4
0	0	0	0	0	0	H02I12.7
0	F	0	0	0	F	H06H21.3
F	0	0	0	0	0	H06I04.i
0	0	0	0	0	F	H19M22.1
0	0	0	0	0	0	H23L24.c
0	0	0	0	0	0	K03A1.1
0	0	0	F	0	F	K05F1.5
0	0	0	0	0	F	K12D12.2
0	0	0	0	0	0	R05D11.3
0	0	F	F	0	F	R08D7.1
0	ND	0	0	0	F	R11D1.8
0	0	0	0	0	0	T01C3.6
0	0	0	0	0	F	T02G5.9
0	ND	F	F	F	F	T03F7.5
0	0	0	0	0	0	T10C6.11
0	0	0	F	0	0	T10C6.12
0	0	0	0	0	0	T10C6.13
0	0	0	0	0	0	T13H5.4
0	0	0	0	F	F	T23B12.2
0	0	0	0	0	0	T28F3.2
0	0	0	0	0	0	VW02B12L.1
0	F	F	0	0	F	W07E6.1
0	0	0	0	0	0	Y106G6.H.3
0	0	0	0	0	0	Y41D4A_3073.a
0	0	0	0	0	0	Y41D4A_3457.a
0	0	0	0	0	0	Y41D4A_3457.d
0	0	0	0	0	F	Y47D3A.c
0	0	0	0	0	0	Y62E10A.d

0	0	0	0	0	0	Y71G12A_187.b
0	0	0	0	0	0	Y76B12C_66.c
0	F	0	F	0	0	ZK550.4
0	0	0	F	0	0	ZK637.8
0	0	0	0	0	0	ZK652.1
0	0	0	F	0	F	ZK686.1
0	F	F	F	F	F	B0454.1
0	0	0	0	0	F	B0491.5
0	0	F	0	0	0	C02F12.8
0	0	0	0	0	0	C06A1.1
0	F	F	F	F	F	C10A4.4
0		0	0	0	0	C14C10.3
0	0	0	0	0	F	C15H9.4
0	0	0	0	F	0	C16D9.5
0	0	0	F	0	F	C18E9.4
0	0	0	F	0	F	C29H12.6
0	0	0	0	0	0	C30B5.6
0	0	0	F	0	F	C30C11.2
0	0	F	0	0	F	C37H5.5
0	F	F	F	F	F	C40D2.2
0	0	0	0	0	0	C42C1.3
0	F	F	F	F	F	C47C12.2
	F	F	F		F	D1054.3
0	F	F	F	0	F	F08D12.7
0	0	0	0	0	F	F19F10.9
0	0	0	0	0	0	F26A1.10
0	0	0	0	0	0	F29C4.2
0	F	F	F	0	0	F32E10.1
0	0	0	0	0	0	F33A8.1
0	F	F	F	F	F	F40H3.1
0	F	F	F	0	F	F45C12.7
0	F	0	0	0	0	F45H10.4
0	F	F	0	0	F	F46C8.1
0	F	F	F	0	F	F47F6.4
0	0	0	0	0	0	F52C6.13
0	F	F	F	0	F	F54F2.7
0	F	F	F	F	0	F55C12.2
ND	ND	0	ND	0	ND	F57G9.3
ND	ND	F	ND	F	ND	F57G9.4
0	0	0	F	F	0	H06I04.h
0	0	0	0	0	0	K02E7.6
	0	0	0	0	0	K06A4.6
0	0	0	0	0	F	K06A5.4
0	F	F	0	0	F	R07E3.2
0	0	0	0	0	0	R12E2.2
0	0	0	0	0	0	R144.2
0	0	0	0	0	F	T12A2.2
ND	0	F	F	0	F	T19B10.2

0	0	0	0	0	F	W01A8.4
0	F	F	F	0	F	W01B11.5
0	F	F	F	0	0	W02B3.7
0	0	0	0	0	0	W04A4.6
0	0	0	0	0	0	W07B3.2
0	0	0	0	0	0	W10C6.1
0	0	0	0	0	0	Y38F2A_5743.i
0	F	F	0	0	F	Y51H4A.m
0	0	0	0	0	F	Y53C12B.2
0	F	0	F	0	0	Y57A10A.v
0	0	0	0	0	0	Y75B12B.3
0	0	0	0	0	0	Y75B8A.27
0	0	0	F	0	0	ZK121.c
0	0	F	F	F	0	ZK546.2
0	F	0	F	0	F	ZK795.3
0	0	0	0	0	0	C04G2.6
0	0	0	0	0	0	F41H10.7
ND	ND	ND	ND	ND	ND	T10B5.5

- As indicated above, the epistasis analysis may be used to order *C. elegans* genes in a genetic pathway. Mammalian orthologs of *C. elegans* genes are expected to occupy similar positions in the mammalian fat metabolism regulatory pathway. For example, this analysis places *C. elegans lpo-3* gene downstream of *daf-2*, *tph-1*, *lpo-1* and *lpo-6*. Based on this result, it is expected that inactivation of the human *lpo-3* ortholog would cause a reduction in fat content in normal individuals, as well as in obese individuals who have a mutation in a fat storage gene upstream of *lpo-3*.
- Understanding the position of mammalian genes in a fat metabolism pathway facilitates the development of therapeutics for the treatment of obesity and obesity-related disease. Drugs that target downstream fat metabolism regulator gene will likely be effective for the treatment of any fat metabolism regulation disorder, obesity, or obesity-related disease that results from a defect in an upstream gene. The downstream genes identified in *C. elegans* are promising drug targets for the treatment of obesity and obesity-related diseases, such as atherosclerosis and diabetes, or even hyperphagia-related syndromes.

### RNAi Screens in Sensitized Genetic Backgrounds

In mammals, activation of the sterol responsive transcription factor, SREBP, depends on its cleavage by two proteases that liberate the transcription factor from the lipid membrane. In *C.*

*elegans*, RNAi of SREBP resulted in a reduced fat phenotype. RNAi of *C. elegans* site 1 and site 2 proteases, however, failed to affect the fat content of wild-type nematodes. Interestingly, when RNAi of these proteases was carried out in *lpo-1* (VLDL-R) mutant nematodes, a reduction in the fat content of the mutant nematodes was identified. Similarly, RNAi of the 5 nematode long chain fatty acid transporter (D1009.1), sterol carrier proteins, SCP1 and SCP2 (D2013.8 & ZK892.2), and ERG-3 like sterol desaturase (F49E12.10) decreased *lpo-1* mutant nematode fat content, but had no effect on wild-type nematodes. Thus, the high fat content of *lpo-1* provides a sensitized background for the identification of fat metabolism regulator genes that cannot be detected in a wild-type background. Systematic RNAi of the *C. elegans* genome 10 could be carried out in any sensitized background (e.g., *lpo-1*, *lpo-2*, *lpo-3*, *lpo-4*, *lpo-5*, or *lpo-6*) to identify new fat metabolism regulator genes.

### Mammalian Orthologs

The results detailed above indicated that key mechanisms of body fat and sterol 15 regulation were conserved between mammals and *C. elegans*. Thus the powerful genetics and genomics of *C. elegans* can be exploited for the systematic identification of mammalian fat metabolism regulator genes, their interactions, responses to environmental perturbations, and changes over the lifespan of the animal. Importantly, analysis in *C. elegans* provides insights into the dysregulation of energy balance that underlies human obesity-associated diseases. 20 Moreover, the comprehensive RNAi system described herein allows for the rapid identification and classification of new fat metabolism regulator genes in *C. elegans*. Many of these genes have mammalian orthologs not previously associated with fat metabolism. These mammalian genes may be unidentified components of known fat pathways, or present new paradigms for fat balance. Given the regulatory interactions that exist between fat cells and the CNS, the study of 25 fat regulation in a physiologically intact animal, such as *C. elegans*, can provide insights unattainable in other model systems, such as cultured mammalian adipocytes.

Most of the genetic loci identified herein cause a decrease in fat storage when inactivated. Activating mutations in such genes could cause obesity in humans. The mammalian orthologs of 30 these *C. elegans* genes represent candidates for pedigree analysis in obesity and lipodystrophy syndromes. The mammalian orthologs of identified *C. elegans* fat metabolism regulator genes

that decrease *C. elegans* fat content are shown in Tables IX and X. These genes were identified as follows.

Protein sequences corresponding to genes of interest were retrieved from the repositories of *C. elegans* sequence information at the wormbase website and matched against the *C. elegans* orfeome database from the Vidal laboratory (worfdb) hosted by Harvard to ensure the accuracy of known or predicted protein sequences. The protein sequence was then used for standard [BLASTP] searching using the National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health BLAST website. The search was limited to “non-redundant mammalian database.” Translated database [tblastn] searches were performed in the same way except that the [tblastn] search site was used instead of BLASTp. The protein sequence corresponding to the top mammalian candidate produced by tblastn was retrieved from Genbank at the National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health website and used for BLASTp search of *C. elegans* proteins using the wormbase site.

These methods allowed us to identify mammalian orthologs of the worm genes revealed by our genetic or RNAi analysis. An ortholog is a protein that is highly related to a reference sequence.

#### **Mammalian Orthologs of *C. elegans* RNAi Clones that Reduce Fat Content without**

#### **Reducing Viability/Growth**

Mammalian orthologs of *C. elegans* RNAi clones that reduce fat levels without altering growth or viability are shown in Table IX. Such genes are particularly promising therapeutic targets for the treatment of obesity or obesity-related disease, since drugs that inactivate them are unlikely to cause adverse side-effects. This list identifies the *C. elegans* genes by *C. elegans* cosmid name and open reading frame number. The mammalian orthologs are listed by the Genbank protein accession number.

**Table IX. Mammalian Orthologs of RNAi Clones that Reduce Fat Content without Reducing Viability/Growth**

GENE NAME	MAMMALIAN ACCESSION NUMBERS				
C36A4.9	8923896	15082523	20861072	9790021	21269872
AH10.1	13376741	15559516	17390865	16418449	18034773
C17C3.1	14548007	18543371	18875408	14285685	4885565
K05F1.3	17440754	4557231	7542837	8392833	2392312
T08B2.7	595267	14328041	4504325	7387634	18677763
W01C9.4	4503301	18571478	1575000	111287	17105350
T02G5.4	4557237	86728	499158	135757	21450129
F14H8.1	19718741	17529999	17529997	19718746	7662298
F11E6.5	18496985	17454617	10444345	13129088r	16151801
B0285.8	4557455	18603990	6671748	6978649	8393104
Y49A3A.1	19527084	5174415	18580821	9910384	12841853
F23H11.9	10092647	12832369	12835319	12854793	18560903
C01C10.3	15489111	7661996	19353717	12834446	11427110
F08F8.2	90238	123344sp	2495262	123341	4557643
F15A8.6	4388919	108929	3219994	3212451	3401962
K02D3.2	11992399	19525698	19525702	11992401	19526998
M05B5.4	6912484	18265286	19527008	6678672	12836459
K10B3.7	8393418	6679937	2506441	120707	6983849
H04M03.1	130757	13653992	539657	1709731	4505639
F43H9.2	6685594	6755656	4758668	2137762	6691980
Y6B3B.10	137047	11641421	18490663	3264848	13385556
K09D9.2	117254	6166042	117218	10835506	19071838
K07C6.4	117219	2144294	117197	15147326	65684
K07C6.5	2134974	18088282	117225	1168128	117235
T04A8.16	6753258	7656959	4495099	2970661	2584822
F28H6.3	4757928	6318318	2143819	1083706	2144056
C06E7.3	13097429	5174529	19705457	284301	4557737
F13D11.1	8392842	19484058	130728	13111975	4557010
F52B11.2	4557839	12833938	14776420	8393988	18594118
K03B8.3	6678862	285323	6981198	1083414	91080
C24A11.9	18575685	7657653	9507201	12848965	9966853
T09B4.8	13994255	13929196	2492864	1096025	12836724
Y55F3C.c	14548281	2331277	2136328	6755885	2052504
T12A2.1	18579028	12836736	18490737	17511765	7705596
C31H2.3	12659007	14758806	7406615	4468339	18553930
E01A2.i	8393446	6680019	4504011	9588455	556518
C46H11.2	18252634	3171877	4503759	19421731	2494584
M28.6	13507666	14249588	17380287		
F37B12.3	16173159	16171486	13384840	18594817	5835803

C37H5.3	19527302	19353227	11545767	13649744	13385690
ZK6.7	758064	4758676	7546565	6647602	7434997
R07B7.9	3172337	464376	2696236	18599609	12854561
F31F6.7	15277824	18916767	18572895	7513594	17512305
F13D12.6	2098347	4505989	13929457	12653639	3191969
C15B12.7	14141726	12408302	6678017	8922155	20834606
C44E4.6	118276	10140853	118275	493800	13937379
Y47D3B.7	3318712	12249193	12249195	17380503	13543808
C33G8.9	643611	543297	14149746	18158445	6753104
K08A2.b	7446194	1082439	2135340	4504443	9714201
Y69A2A_7278.m No significant similarity found.					
F11C1.6	15290639	11493781	3121738	416584	10945629
B0280.3	6677767	15930194	1350838	18550866	17456433
F11A1.3	8394529	2500921	4507883	13879475	6678561
C46E10.9	18561872	18595962	4505855	9910494	14250235
C47C12.3	11463867	14916471	6677645	6677643	6677647
T09F3.1	16551981	18590023	18546120	14349043	17482343
T23F11.4	9055310	14245734	1663696	14747600	6031194
ZK686.4	13385046	17391140	13507650	18572630	10436199
Y116A8C.32	7513392	1620405	14318588	14165553	7513389
F22A3.4	No significant similarity found.				
C09G9.7	4557827	4557823	110788	417447	280984
W02C12.3	15282044	4557755	1092105	6678884	13124344
F22A3.5	339895	18149007	107390	8567384	14782520
C01G6.5	6624734	631765	13435494	14782132	5420465
F39D8.2	14780600	14043520	4758930	2190414	7710080
R04A9.4	124222	7546552	110568	4503535	6681293
ZK757.3	11386878	11024680	18570004	6912352	13376275
Y41E3.10	12653785	12856949	12845656	12845723	4503477
D2089.2	12836671	19263985	16358983	12805349	7706043
F11A10.3	2327052	13111867	5729939	2687591	5931741
B0218.5	18201865	14781533	13122442	13435470	13097702
T05C12.1	20900385	18201865	20555151	6526520	13122442
Y53C12A.1	19264036	12746438	2914671	4758928	2460023
C16A11.3	110864	125290	4502885	4758008	13623342
F45H7.4	9506973	8393959	16580805	3800869	4505811
ZK930.1	14728229	17028437	12836135	14150114	4507281
ZC504.4	3721838	18553056	3327188	6679060	18555837
M01B12.5	16549132	13899340	12856491	16163884	18152791
C02F4.2	4584820	19343933	8394030	14209665	6715568
C06A1.3	130706	13994195	542987	4506003	1535
ZC302.1	5031921	11560107	5031923	9055282	1401337
C03D6.3	6755342	18042848	14750499	4506563	12833263
T19D2.2	4506283	17528929	4506285	1246236	17865337
C47D12.1	4507691	4151929	14748864	3694663	7512506

ZK909.3	12835118	18584663			
C06A6.1	No significant similarity found.				
R107.4	7661946	9789983	7019547	12835969	9790253
C33H5.17	17939660	14017911	14249740	14042873	18204508
R07E5.1	14763089	13543933	13385692	8922283	12852170
C41D7.2	12853786	4557803	8134591	10945625	8099648
ZK675.1	4506247	6679519	1335864	6679517	4325111
F20H11.2	11990420	7023114	18255692	7662410	16163366
T04D3.2	No significant similarity found.				
C44F1.5	12018268	729241	8176552	284522	423625
H08M01.2	17482677	12697989	8886143	285370	13649298
F46G11.3	12730586	4885251	17375734	13591947	12803719
K10D3.5	14042287	7019333	13435804	15079264	11360161
F41D9.1	14165549	19527318	11034851	7209313	5689447
F07C3.4	7657152	627469	12856817	7305093	7705931
F45E4.6	3108187	4557799	13633370	8705244	9506547
T14E8.3	7381416	17986270	3820492	4503385	11344838
C07A9.2	17449930	16758328	4503837	18570787	17473912
Y4C6A.h	18490394	400254	4504139	6288800	11279202
C38C10.1	107184	7669548	6678213	107185	8394408
C34C6.6	6679581	12084650	9663119	3005920	16197740
E02C12.3	17559196	17559194	17566398	17562326	17564240
H09F14.1	11225272	4557859	464813	464812	7229404
Y44A6B.2	No significant similarity found.				
F58G4.2	17464941	6981586	13358632	14388397	
Y40H7A.7	18567340				
F07C4.1	No significant similarity found.				
F10A3.13	No significant similarity found.				
F17A2.7	No significant similarity found.				
F47C12.3	4505807	1944499	7547268	17105400	2136690
F49C5.6	No significant similarity found.				
T07C12.1	18556170	5834956			
T07C12.5	11023094	6755913	66358	2117664	136595
Y17G9A.d	No significant similarity found.				
Y94A7B.3	No significant similarity found.				
Y9C9A_53.c	No significant similarity found.				
T04A11.8	4115532	4959453	7159263	7159275	7159273
F33G12.2	14150114	18044039	12838548	13385884	13542766
C32C4.1	16758912	4758622	13242172	15418950	5921784
B0310.1	4504851	4504849	14149764	11496265	13124041
C37A5.1	4759310	18044531	8923137	3335161	18476496
K04E7.2	2143888	2832268	1136776	4827008	15301458
C34G6.4	6755046	6755048	266517	2506118	307180

K05F1.6	4506999	8918937	2511670	4507005	15147378
ZK682.2	9719374	6912666	9719376	4885441	15487160
C13D9.7	18875376	13376449	17865509	17865499	12597441
F23F1.6	6671596	18181964	6981556	539952	1706187
F15H10.4	17505223	18426842	17473038	12005633	14767497
F59F5.1	7513431	5730045	6677997	4759116	4759112
C46F11.1	3355534	10092641	18564398	17390915	13569893
H27A22.1	12856921	18043218	6912618	2498823	12841414
T19B4.6	18203852				
T19B4.7	4885175	6681139	6978755	4505375	2078518
T27F7.1	6716764	7706353	12852884	12844196	12858406
C33A12.1	400650	4826848	14777313	12844560	6981260
F28H6.2	3336980	12839964	1083460	6753746	3024410
F20D1.9	12845461	13375983	13899342	12837673	18848167
F14D12.2	17462167	10433878	18860509	13542844	14789889
K12B6.8	7662244	17068424	18590847	14149702	14789737
C15H9.7	16758776	2143818	12852371	4504937	12654129
W03C9.3	13027392	14746535	131797	13794267	6679599
F11A5.3	18088786	12837642	10946940	13929006	108107
R11A5.1	4501975	18201935	15929245	20909238	6753076
F53H8.1	9055268	6912240	19424296	12836141	10439979
T14D7.3	12851438	7949160	14736500	4507863	4200247
R01H2.3	4507157	8928391	8928392	7513715	7513686
T22D1.4	4506675	19527152	9857227	14124942	6981486
F54H5.3	8099350	12842294	14759532	4507867	7305623
C05E11.2	No significant similarity found.				
K09B11.9	16359203	13431959	1171952	9507177	1171950
Y38E10A.c	8925888	8925890	7513043	4758956	14769776
C49C3.3	No significant similarity found.				
F49E12.4	4507789	10444495	8134778	4759282	18582039
F52C6.2	No significant similarity found.				
Y65B4B_10.a	No significant similarity found.				
Y65B4B_10.e	6680476	13554239	15079474	14010859	18554931
F40H3.5	14336772	4826764	14494990	20899626	9957244
ZK39.7	12742008	4758084	6681063	13928904	3288885
F49E11.4	13899332	18490353	6678423	14042040	15779065
K02D7.3	No significant similarity found.				
M01E10.2	2065167	18201921	3182940	18201923	14757167
Y77E11A_3443.i	19343543				
F07A5.1	No significant similarity found.				
F26D11.10	16418341	6572227	16418453	8393886	
Y37D8A.1	9790141	5031597	2209347	18104938	13639437
Y17G7B.15	17434817	16945966	12697977	7661880	18551362
R107.6	7513102	14726914	7513045	16758540	12839687

C23F12.1	7677526	14744078	4557597	5419655	8885790
M106.5	11131728	13124696	1345668	4826659	19352984
C06G3.2	9910266	14424665	13194197	3891936	9910292
T28D6.2	135395	223556	135412	18601895	6755901
W09D10.3	4506673	11177148	12837801	17865556	12832973
W10D9.5	12842129	20902823	9910382	20841046	17475714
F54D5.11	4504195	13386074	18043581	228300	11611843
F44B9.7	12847646	6677723	4506491	283966	13097123
Y71H10B.1	6912598	2668557	12841987	18544261	7706749
F21D5.5	14211141	6005836	5757919	12803393	14786918
F20D12.2	11225270	19923191	7513026	13938593	14780851
Y41D4A_3073.a	4758844	6093462	18875386	16758834	7512639
T22D1.10	5730023	6755382	4929561	9790083	4506753
C24A1.4	1263081	1585320	14286268	3005702	5730039
Y37A1A.1	3005702	5730039	1263081	14286268	1585320
W09G3.4	11056006	18546470	14744278	14602983	12846468
H25K10.1	164720	130722	178006	13016699	7767180
C30F12.1	18604558	13376632	14750657	2224587	16041792
W09G3.1	18582801	7662058	18578718	6005796	14388374
Y51H4A.m	19483873	20985890	14760337		
Y48C3A.b	13507702	18558641	13507706		
F13E6.1	13385202	4507643	11125673	15723372	17450493
T21D12.3	9506989	16307140	5031957	11125356	10801584
B0041.5	17391272	12836420	10437002	13376774	4758846
B0286.4	11640600	7657385	12847218	7020899	6841484
D1054.14	20841107	20472100	20881439	14249602	14149989
F54C9.9	14602715	12804075	10438567	12711672	13649858
B0513.7	3005702	5730039	1263081	14286268	1585320
Y41D4A_3192.a	12052882	8922808	12849037	18548855	16550576
ZK686.3	14714487	6166601	1353701	6996934	14149775
B0041.3	12843216	14585867	9651081	12847693	13385022
C05E11.1	14725463	12697975	16551959	15777193	15843561
F38A5.1	18559316	14714703	8922938	13529584	13899211
C07E3.2	18550740	10434347	18598995	8480755	7512721
Y47G6A_245.b	11360017	5689555	14725071	11360104	18544047
R05F9.8	8099350	14759532	4507867	12842294	13928870
W04A4.5	14124974	16553697	18566093	15809018	14602672
Y49F6B.n	6919955	15278160	15928532	14767139	5901878
F14D2.4	18556673	13528918	12314036	13627447	7656845
C30G4.5	No significant similarity found.				
F58H1.6	6912322	18875406	18175295	17464187	18175284
F55B11.4	18602286	20557594	20825014	5419655	14744078
C47D12.7	13431657	6329805	8393672	6644293	18490684
ZK355.d	18490618	458124	1352359	1071851	10880776

C16C4.2	no similarity				
Y50D7_165.b	No significant similarity found.				
C54H2.5	6755698	19557691	2414516	5531849	18571517
T05F1.6	20151156	2135874	5032189	16754836	20911529
R08F11.2	8567364				
W01B11.5	No significant similarity found.				
R05H11.1	2498010	8659574	1524099	107912	6470339
C32D5.11	4504867	13645747	18255721	13905224	12585549
H32C10.3	9506623	4589536	14755049	6841238	3329427
T21C9.2	7023936	15297473	16518394	8980452	7705397
Y41E3.11	12803479	14786840	5901942	18204832	16041796
T10D4.1	28880	402621	556809	7435163	114258
C54G7.1	18581301	13872241			
Y57G11C.17	14198202	8922621	4826524	14149736	18490309
T04C10.2	7019369	3894395	11177890	10433856	16923990
W06G6.1	18588797	11342591	5453998	11544639	
K12D12.4	12849906	15277327	15777917		
C23H3.2	9955966	16878304	9845230	11560034	9588087
C56E10.3	4758200	3702136	2134996	14781619	181608
T27E4.6	18588797	3023767			
Y57A10A.bb	18599579	13874598	1235902	4502325	
C14A6.6	13654761	4502265	14779030		
Y67D8A_380.d	11611581	7022736			
F08G2.7	No significant similarity found.				
F29B9.11	8843925	3023219	8843927	409029	
T26E4.13	18604070				
H04M03.4	92459	13236528	226416	87490	6680904
K02E7.11	16553058	18590066	17026040	14210297	2344954
T10C6.10	16758598	2623539	7661820		
Y57E12_242.c	16041755	1754827	13787219	7671650	1403336
T10E9.6	18043543	13623239	182403	18560910	17443348
Y111B2C.e	8925872	6753636			
Y71H2_389.a	17433405				
R160.4	4507575	339760	8923171		
Y50E8.q	1872417	6907077	951291	2291076	2291068
F13B6.1	6678561	13879475	8394529	4557543	
T21C9.11	18548863	18557544			
T02H6.7	6978493	1346465	729945	17505210	16716337
F53A9.4	18552947	18028934	18028932	11934691	11641259
C14F5.3	2148107	4507627	2144843	15072321	1717775
C29H12.6	20341671	3661610	7023218	20149742	19745190
C56E6.4	17459650	15072441	16933555	14581464	18583597

F46C8.7	2499095	9507113	2499094	18543351	
F46F5.10	14589929	10047189	18485486	14773966	11360033
C42C1.6	18561804	18702327	19684085	17469647	
F27C1.4	13386030	12856989	6981448	7706123	16758970
B0554.7	13236549	2708503	2708511	2708501	
C18E9.5	4507691	7512506	4151929		
F28H7.6	6685708	8101764	8393978		
C08G5.2	gbAAL29692.1	gbAAC32740.1	gbAAA36456.1	dbjBAA83105.1	NP_033853.1
F54F7.2	4557385	1082640	5453774	506818	14720600
M01A8.1	6753760	17225492	7650140	13492036	1708335
T01D3.4	13027456	14041800	14765556	16716571	14211847
F59E11.5	16751835				
T19D7.1	18640740	9938026	11120504	11559956	5174469
B0554.6	18875378	16877798			
ZC84.5	7706431	9790043	17457573	2499057	18157547
W05E10.2	18552761	19527020	20072462	14784755	8923510
ZK652.2	4468341	9506859	12834792	13643685	
D2062.10	10946736	5442366	13431270	14917111	
C17G10.7	4885525	13623360			
B0207.9	6686100	11056036			
F38E9.4	7427513	18555663	9506701	18561409	
Y65B4B_13.b	6643819	17441938	185364	6643719	5454100
Y38E10A.b	16758026	14210276	17448743	6331226	14758637
ZK593.3	126363	34226			
C14C6.8	15307762	13899227	9055260		
F21H12.3	5453607	2947308	6679517	4325111	4588015
T23E1.1	12805635	6981448	4505111	180654	18588157
T17H7.1	110087	18581352			
F10A3.11	5902154	4586880	6552408	6552404	
F41C6.6	16758936	4587083	18599218	5006891	5032101
T07C12.11	4885603	10443498	17368511	18572506	10880987
T14A8.1	6850974	18044375	12731793	8922436	
F58F9.1	1703342	6680706	18548973		
ZK154.4	19421557	11345539	14133197	7657697	
R03H10.4	14763809	6686295	4505263	5032003	6755246
F55C12.3	4757720	10862692			
Y119D3_456.a	6678922	5533377	7243011	7514128	6760665
F08D12.4	No significant similarity found.				
R11H6.6	No significant similarity found.				
Y57A10A.I	No significant similarity found				
C14A4.12	No significant similarity found.				

Y7A9C.3	No significant similarity found.				
C01G6.9	No significant similarity found.				
Y51H7C_255.c	No significant similarity found.				
C15C7.5	No significant similarity found.				
T10C6.4	No significant similarity found.				
Y37D8A.8	No significant similarity found.				
C50E10.5	No significant similarity found.				
ZK1290.1	No significant similarity found.				
F22E5.1	16552612	17976800	13994213	17976802	
F12A10.8	No significant similarity found.				
B0034.2	No significant similarity found.				
F36H12.15	No significant similarity found.				
F52C6.12	20841107	20472100	20881439	14249602	14149989
Y24D9A.b	No significant similarity found.				
K06B4.3	No significant similarity found.				
T11F9.10	No significant similarity found.				
T19D2.3	No significant similarity found.				
T27E4.7	No significant similarity found.				
Y105E8B.a	No significant similarity found.				
Y69A2A_7278.l	1843522	7446193	2135340	9714201	6166208
Y51H7B_5.b	No significant similarity found.				
T13F2.6	No significant similarity found.				
K09H11.2	No significant similarity found.				
T26E4.10	No significant similarity found.				
T06H11.2	No significant similarity found.				
F59F5.2	No significant similarity found.				
ZK131.8	12854993	12847763	4504301	70762	223582

#### **Mammalian Orthologs of *C. elegans* RNAi clones that Reduce Fat and Reduce Viability/Growth**

Mammalian orthologs of *C. elegans* RNAi clones that reduce fat content but adversely affect nematode growth or viability are shown in Table X. This list identifies the *C. elegans* genes by *C. elegans* cosmid name and open reading frame number. The mammalian orthologs are listed by the Genbank protein accession number.

**Table X. Mammalian Orthologs of *C. elegans* RNAi Clones that Reduce Fat and Reduce Viability/Growth**

<i>C. elegans</i> Gene	Mammalian Accession Numbers				
F10D2.9	13929208	91311	13938635	6677861	13277368
F29D11.1	13562118	6806919	15825096	4758686	15825005
F32H2.5	2506136	8394158	204099	9937097	7433799
W06D12.3	12698736	13431283	13431274	14388941	11360339
w09b6.1	542750	20559521	13626188	3023534	16758804
C09H10.3	19526814	163412	17472883	14198176	548387
C53B7.4	15929392	5453561	7513261	2493093	12841973
F02E8.1	19705465	114617	12833323	13637998	4502299
F35G12.10	19705465	114617	12833323	13637998	4502299
F37E3.1	4505343	627397	15988381	5441937	12851646
W04A8.7	29733	2137085	2136308	4759206	6680936
Y110A7A.h	162703	4502311	549205	12643271	17105370
Y37D8A.14	6680986	12858580	117100	18999392	4758038
Y57G11C.12	4505359	400384	13385492		
F28B3.1	17471893	6686133	14043353	17457389	6686101
C23H3.4	6678125	2853289	6685595	5454084	2136140
C42C1.5					
D10I4.1	4502241	1399961	15559708	6005990	14766617
E01A2.i	8393446	6680019	4504011	9588455	556518
E04A4.7	229351	118007	118014	117994	117966
F01G10.1	12855432	388891	12018252	4507521	1729977
F40H3.5	14336772	4826764	14494990	9957244	6754246
F44D12.4	18203658	16758060	5031715	9055336	18656362
F46E10.1	17390865	13376741	15559516	16553412	4164168
F57B9.2	4589658	12653967	18598275	19354215	7706214
H14A12.2	8393358	227665	68293	13543801	12832319
H15N14.2	20913355	13489067	6679140	134267	90219
K02F2..2	20892597	9951915	7709980	178277	13096485
K06A4.5	17921976	6912406	9910256	13637831	4433351
T05H4.4	1709233	1070443	4503327	19421846	553600
T05H4.5	127847	1070444	17943396	1709233	19745150
Y55F3A_750.e	12841560	6755911	16758644	4759274	7949156
B0285.1	14110390	14110387	7706549	4240297	14748750
C16C2.3	1352493	9966773	12836107	13249985	346209
F10E9.7	11137114	6978573	18588573	186083	1699382
W03F8.5	4504951	293690	126367	226290	6981142
W07E6.2	8922428	12804063	17390943	3043443	7305363
ZK1067.1	4503597	280818	119534	17432904	10880776
ZK675.1	4506247	6679519	1335864	6679517	4325111
C33D3.1	8648977	3123218	477102	15593990	5882288
C34H3.a	16506291	17298684	16877372	6754928	19344028
D1081.2	14719546	18655799	10048414	4507205	7546482
F10C1.5	13940223	18572015	11230443	12229781	11386173
F22A3.1	6912580	7305415	18204910	15214598	4557551
F23B12.7	1705659	5031625	6753402	10439934	10434534
F25H8.3	13626125	7242979	9910122	7243073	6685072
W01D2.2	9453875	1843522	2135340	4504443	2135341
Y17G7A.2	18027804	11870006	18602376	6729087	19173808

C01F6.8	12848135	2197127	1095482	7513563	1060971
C56C10.8	17441804	2851417	115143	107909	29507
C56E6.1	11967969	16758590	14141178	16158955	15146444
W06D12.2	16306555	11560129	11177516	19483870	13124041
W10D9.5	12842129	12844929	9910382	17475714	16930809
Y61A9LA_75.a	7661980	18575034	14737076	18576250	7243183
ZK105.e	12408294	205634	17149816	18203577	18202498
C36B1.4	4506189	7106389	4092058	18577124	1346784
CD4.6	9910833	8394060	4506179	14768212	13543551
F23F12.6	2492517	5729991	1172637	3450955	7110701
F39H11.5	3914439	14198355	3915806	16165126	13928866
T23F2.1	12846285	14861836	9910440	12836608	18572937
Y38A8.2	4506197	11424309	6755202	8394082	17447021
C36E8.5	7106439	135490	5174735	14758306	12846758
D2024.6	12841166	5453597	16740716	1345694	6671672
F10C1.2	34228	125962	5031875	1072002	383110 .
F20G4.3	13928704	1346640	13431706	17978023	12667788
F44F4.11	6678469	14389309	135395	90217	135412
K07C5.1	5031571	15778930	14769120	12852068	1351867
T04C12.5	71621	4501885	16304154	1351867	16359158
Y19D2B.1	6678469	12850141	12839396	90217	2843123
ZK593.5	13259510	4139121	1419567	13259508	6681147
B0303.9	18105056	12621146	12859683	7514114	10439792
C02C6.1	6681207	729381	1083647	18093102	539580
C05D11.2	19343731	15553046	17978479	11345382	13385360
F29G9.3	12005732	4506957	12837633	4557471	5630084
F41C3.4	7634779	7705636	13385354	20340619	17488855
K02D10.5	16758654	6685966	12836691	12963651	4759154
T21E12.4	9506549	13384736	729378	18582791	2224591
B0222.6	NF				
F57B9.5	17028426	13938404	15011984	15208645	3929339
K01A6.4	NF				
K12D12.3	18780273	12052774	17974510	18563487	5102636
W10C4.b	12857852	4506217	20532221	20985633	8394085
B0035.7	631691	17455198	18545931r	1458139	18580602
B0035.8	223096	280961	18564726	4504263	15030326
B0035.9	12854993	12847763	4504301	70762	223582
B0041.4	2500343	11968086	16579885	12846949	1363989
B0495.6	18585599	1931584	4503525	19263839	19354442
C03C10.3	4557845	7106399	2500209	11256408	14743689
C04H5.6	14318701	14250712	14752410	4503293	7770157
C06A8.2	4507101	7243201			
C08B11.5	5032069	18582960	18582878	4504715	17489281
C09H10.2	4506651	14750580	13645150	9845295	17472837
C15F1.e	19424322	17511746	10436247	9966779	10433787
C15H11.9	3183219	14719402	10946930	12857046	12845436
C16A3.3	12834845	2498864	17454886	4454542	13385288
C16A3.4	15529978	8922413	10435029	18583383	12698069
C16A3.6	14042167	14210516	14747081	15341814	12853682

C26D10.1	4502801	14278207	4389390	87057	132171
C26F1.9	18592185	18590969	4506647	14738021	17449824
C27F2.4	12846835	16226067	13384748	12843473	12652833
C29F5.3	7662204	4521188	14750657	2224587	16041792
C37H5.8	16158324	12653415	6754256	1072476	4758570
C42D4.8	5902062	133327	2145091	7434727	4505939
C47D12.6	14861852	12653491	14714853	12845562	4507367
C50F4.5	223096	280961	18564726	15030326	16306566
C52A11.2	338669				
C52E4.3	4759158	17471847	2833357	12862083	7657315
D100T.6	14195014	14782930	13540714	13639605	14195007
F09E8.3	4505253	3108220	5725250	12655021	3986757
F09F7.3	4505941	7022241	8922399	6677789	3005758
F18A1.5	4506583	18390321	12860240	2624702	13096131
F20D12.4	15214617	4759344	11387254	18546147	126369
F22B3.1	12854993	12847763	4504301	70762	223582
F22B5.2	8393308	6492222	2460200	4503517	14280325
F22B5.9	15296128	5032011	6841566	12845588	12644592
F26F4.10	586063	18043638	15149476	12847471	1711647
F26F4.11	14589953	1710659	4406232	476961	6680928
F32E10.4	6680598	4504901	6680596	14758897	2654139
F37C12.11	4506699	17390310	12841661	13592073	12964241
F37C12.9	5032051	12083607	10181112	7440317	16158168
F45E12.3	11140811	13270467	13259127	16307345	13386300
F45F2.13	4504281	4504299	386772	18595043	70749
F54E12.1	4504281	4504299	386772	18595043	70749
F54E12.5	631691	17455198	121983	8922758	18545931
F55C5.8	7657617	134889	7513444	18044248	14041927
F55F10.1	7305641	14250229	17459629	6981606	1655681
F55F10.2	7529573	17512348	4678973	15029526	18604727
F55G1.10	631691	17455198	18545931	1458139	18580602
F58A4.4	6679459	110830	3676248	4506051	12847590
H02I12.7	631691	17455198	18545931	1458139	18580602
H06H21.3	4758254	12859663	11418342	4503499	3746340
H06I04.i	18028291	17017991	13384672	15126717	7019917
H19M22.1	18676514	18702313	7959295	9507013	18660896
H23L24.c	11024694	17480509	18587523	18572403	12853018
K03A1.1	7305139	70743	70749	4504281	2119013
K05F1.5	8923303	18070860	18545138	18070859	14762794
K12D12.2	18566874	1504030	12852259	17646641	
R05D11.3	5031985	2914436	2780953	7246005	17451119
R08D7.1	14249338	3337385	19344062	17446135r	18565781
R11D1.8	13904866	14603467	1173012	12083655	6677779
T01C3.6	4506691	18549572	18591367	7305445	70920
T02G5.9	16716381	586059	11095909	505108	2501023
T03F7.5	4104236gb				
T10C6.11	223096	280961	18564726	87672	4504263
T10C6.12	631691	17455198	121983	8922758	18545931

T10C6.13	4504281	4504299	386772	18595043	70749
T13H5.4	1082801	18202846	5803167	12854243	18578493
T23B12.2	14756630	12832465	12653925	7705722	9956063
T28F3.2	18565400	13242237	5729877	1708309	347019
VW02B12L.1	12643966	12025532	7329154	13928826	3915315
W07E6.1	189422	5453792	14784289	477430	12653741
Y106G6H.3	4506631	17463853	17435581	18561308	18087841
Y41D4A_3073.a	14603084	18875386	16758834	6093462	6678764
Y41D4A_3457.a	15011027	14249612	9280134	15299647	
Y41D4A_3457.d	4758844	6093462	18875386	16758834	18559983
Y47D3A.c	6679409	6015013	15858951	118838	479803
Y62E10A.d	18561782	133063	12849327	1173072	4506671
Y71G12A_187.b	5901998	4507131	14755615	4507133	13644938
Y76B12C_66.c	18570089	1706101	16878041	16751835	9558725
ZK550.4	11432489	5031727	12847734		
ZK637.8	12643966	3955096	12644129	6755799	8392941
ZK652.1	14755615	4507131	5901998	4507133	11138539
ZK686.1	4506631	7661954	10048468	10442822	6331420
B0454.1	13385706	9506389	5729742	18543825	12643413
B0491.5	14249736	15620873	17865712	18581996	18916890
C02F12.8	14743070	6754316	14579296	15620831	
C06A1.1	2144498	17865351	6005942	6678559	1174636
C10A4.4	18571651	17474970	15546041		
C14C10.3	4826688				
C15H9.4	14916851	14753980	14916847	14724805	13874437
C16D9.5	2498884				
C18E9.4	400383				
C29H12.6	7023218	17438790	18043079	18568537	14211703
C30B5.6	12834595	14250466	18605027	7706047	12856705
C30C11.2	15310156	16550621	4506229	12652653	15126760
C37H5.5	18482381	18389431	10434534	7208452	10439934
C40D2.2	3115347	1770454	3115346	12230855	17465991
C42C1.3	4505531	129309	17459136	2055435	
C47C12.2	No significant similarity found.				
D1054.3	12846547	12841721	12861014	5730041	18567662
F08D12.7	19527308	4557445			
F19F10.9	2342526	8394236	10863889	4427065	13928810
F26A1.10	No similarity				
F29C4.2	No similarity				
F32E10.1	13477303	13430872	17437341	10436236	14010904
F33A8.1	10047283	10438214	13385386	10439972	14727768
F40H3.1	13276695	16551785	12848994	9055326	6624092
F45C12.7	4557445	19527308	13699242	8922617	5174665
F45H10.4	No similarity				
F46C8.1	No similarity				
F47F6.4	7657291	6678762	14043211	18598633	281042
F52C6.13	18548934	6706620	16306483	18643950	18643952
F54F2.7	8923431	17470330	7513001	13446227	14010849

F55C12.2	No similarity				
F57G9.3	1168245	547221	4501959	631067	345733
F57G9.4	19071455	19071447	20836029	18700000	15488938
H06I04.h	No similarity				
K02E7.6	4502853	15823648	13938619	14763181	18157651
K06A4.6	No similarity				
K06A5.4	15620865	18597077			
R07E3.2	No similarity found				
R12E2.2	7705322	13638924	7656940	11359862	
R144.2	13431763	4240137	7706224	13435542	14603356
T12A2.2	12858636	13639549	7513277	6680498	4504787
T19B10.2	4506521	9957313	14774724	6755320	14915764
W01A8.4	No similarity				
W01B11.5	6679661				
W02B3.7	10946676	12858334	18553555	5921784	18490468
W04A4.6	2134794	7657510	18308012	10047205	
W10C6.1	12056971	10435855	6678834	18550879	7804450
Y38F2A_5743.i	No similarity				
Y51H4A.m	8923726	4153862	18568744	7021918	12841382
Y53C12B.2	17390336	10047140	13384846	12844974	
Y57A10A.v	9087217	14719825	7671639	539698	7106435
Y75B12B.3	13569852	18676446	12382773	16507952	
Y75B8A.27	6681147	1419567	13259510	4139121	13259508
ZK121.c	7662442	7661874	15149484	18599919	11360196
ZK546.2	12834355	18597991	12833443	12859847	12849514
ZK795.3	12835200	12850634	18478512	15529982	12845999
C04G2.6	18582324	19923416	7451876	7674415	7512650
F41H10.7	17454617	20137972	18496985	12836437	16151801
T10B5.5	6671704	5453607	12848801	1800303	13540473

#### Mammalian orthologs of *C. elegans* RNAi clones that Increase Fat Content

Mammalian orthologs of *C. elegans* RNAi clones that increase *C. elegans* fat content are shown in Table XI. This list identifies the *C. elegans* genes by *C. elegans* cosmid name and open reading frame number. The mammalian orthologs are listed by the Genbank protein accession number.

**Table XI. Mammalian Orthologs of *C. elegans* Increased Fat Content Genes**

<i>C. elegans</i> Gene	Mammalian Accession Numbers				
C33A12.6	549160	6537138	19527110	5803213	18308170
E04F6.3	4504505	499340	20878362	6680287	13242303
E04F6.6	20896611	12857271	4505257	18602081	
F15B9.5	15529992	7435611	15029967	12840914	17487950
F28F8.2	17390865	13376741	15559516	5019275	13517077
F47B8.3	6840947	5730104	14738770	15680089	17447529
VF13D12L.1	7022512	7705558	11493904	12963757	11281329
C37F5.1	7767065	4100456	535923	14758312	4885201
C43H6.8	2498013	5031945	6754852	5031943	16758982
C56C10.10	4502009	1765936	7709982	6225016	8248030
C56E10.4	5902068	6677829	285012	2780196	386369
F16B4.9	6681852	12803755	5453940	346286	1144348
F33D4.1	15418805	12230055	12230056	5821726	2500908
H12C20.3	12230056	2134678	2500908	4503603	15301634
K10C3.6	7657395	6681852	6680239	11559939	227511
R11H6.5	20534593	4758602	13385872	6855637	13278459
C04G2.2	20900385	20555151	18201865	7949025	20149530
C09G5.8	4589654	20888031	9965252	9966409	9965248
C18H9.7	20839618	6677669	15619013	20178328	631056
C24F3.2	6005956	11560052	12963553	12835696	13435759
F39B1.1	11259849	20843206	20561002	4505799	6755058
F46C5.6	10047321	17402886	12858102	1702997	11386167
F56D5.9	1695739	2642034	2143944	7513459	13647589
F56H11.6	18201865	14781533	5579454	547767	11545751
K08F8.1	10863901	4758700	14589904	1346538	1346539
R10D12.10	18201865	14781533	1311054	1346368	20149530
T04B2.2	6679773	1673620	6003683	4885231	4503687
T04C9.1	7662208	6433901	14587851	13386454	20340540
W03A5.4	19923689	19923274	7514059	1857137	1857139
W08D2.1	17402916	5020354	16716413	17402914	13518017
Y11D7A.9	7657102	6606290	7512548	14602865	16758956
ZC513.1	14583090	18377358	2497615	13929458	5453914
C43H6.9	3935134	2598978	1169965	4504119	3287976
F08H9.5	20903753	6492289	4557503	20473663	14388673
F56B6.5	6981586	6678043	5912550	2136182	4557865
T19D12.8	13938199	13375695	12862152	12845041	12805161
Y27F2A.g	20070376	17457778			
Y40H7A.1	NF				
Y46H3C_11.b	NF				
F32B6.9	4759310	8923137	18044531	3335161	18476496
ZC410.4	9988112	14149764	18652258	11496265	4504851
C18H9.5	9719374	6912666	9719376	18252796	16758166
F14E5.1	687622	8394301	121750	20301952	6755550
F52H2.2	4507053	6103627	3970791	7106415	6319236

C04G2.4	13928870	7305623	6671046	8099350	20070156
F32B6.6	8099350	14759532	3320446	12842294	13928870
C15A11.3	19070657	11907926	11934950	11907928	11934951
C34F6.3	3236370	420194	18204626	17149807	18568576
C53B4.5	5730019				
EGAP7.1	NF				
F46C8.6	14209684	20875879	12849231	177924	2493785
T14B4.7	NF				
T28C6.6	NF				
Y38F1A.9	14211895	14748249	3928489	7513113	7657361
Y41E3.2	NF				
K02D7.5	6677733	12852065	20872938	13543580	10047124
C04G2.5	14042155	17017991	15126717	13384672	
C09G12.5	631119	4758056	2996629	4321116	19547885
C14A4.1	19527182	13775228	20862789	3482908	16306483
C24F3.1a	401208	7657655	13774095	18875432	18202920
C33A12.14	204157	204158	204156	20849315	
C36A4.5	8176554	231629	4502771	19527136	7512404
C44E4.5	14042905	12654293	14718862	14745963	20902053
C50C10.4	NF				
C50D2.1	NF				
D1007.5	20892497	8922756	20845045	10435222	13385900
F12E12.h	5689473	7662364	8574032	17472155	8217421
F25G6.9	16551945	6330933	14250922	13644653	15823631
F25H8.1	18381001	14724179	4454968	18572769	18043204
F25H8.2	19923911	14734590	2506778		
F25H8.5	NF				
F26H9.4	20893587	20536806	14029540	3413920	12620200
F31F6.2	17445681	9651075			
F42G8.5	NF				
F44D12.7	21040405	12845958	20986214	20071715	20878200
F49C12.15	NF				
F49F1.4	NF				
F52C12.2b	20899132	21489947	18598168	11359878	12834488
F56B3.2	NF				
F56F3.4	14741660	11275984	20831147	6677605	5174755
H05L03.3	NF				
H05L14.2	7705835	10944884	13124522	7022528	15426504
K01G5.8a	21165518	13385950	7512937	7661696	2499753
K02E10.3	NF				
K02E10.5	14336702	18585356			
K07A1.13	14789674	6681103			
K07E8.3	6912462	16758422	13811697	18583767	18546498
K09C4.5	19526426	19526424	9910554	5902090	17511906
LLC1.2	18570489	13376713			
M70.1	5757675				
M70.3	NF				

R07A4.2	11056046	15636798	1483238	16716503	6981128
R105.1	12728973	12803719	7662462		
T01C1.2	13385288	17462966	12643822	13876382	9716884
T02C5.3	13928706	72067	20825059	14575679	14767192
T04C9.2	NF				
T05E8.2	7106329	9229937	13561418	13994374	16923261
T07F8.1	6679048	11424724	4885513	13629976	13592049
T12A2.5	NF				
T12B5.8	6093441	105949	19923219	107355	11385307
T14B1.1	6679391	8923793	18582525	16758990	12841832
T14B4.8	9837158				
T14F9.4	12805039	17511715	10047169		
T19D12.3	9055194				
T27A8.4	2906008	7662466			
W06H12.1	13236593	20454983	20521730	16307459	20864376
Y11D7A.8	20860387	20070702	20824271	2497573	556789
Y47D9A.e	NF				
Y57A10B.I	12836671	19584503	8923613	7513036	19263985
Y5H2B.e	17472322	6644328			
Y67A6A.1	NF				
Y73C8C.4	12698099	9966809	14042659		
ZC64.2	NF				
ZK1320.10	20886891	20561771	19743877	132518	
ZK1321.1	4585572	4759146	4151205	20073051	6680427
ZK666.10	18567302				

### Novel Genes Not Previously Associated with Fat Content Reduction

Tables XII and XIII show *C. elegans* genes, and their mammalian orthologs, not previously shown to reduce fat content when inactivated. This list identifies the *C. elegans* genes by *C. elegans* cosmid name and open reading frame number. The mammalian orthologs are listed by the Genbank protein accession number.

**Table XII. Novel Genes that Reduce Fat Content when Inactivated without Affecting Viability**

<i>C. elegans</i> Gene	Mammalian Accession Numbers				
AH10.1	13376741	15559516	17390865		
C17C3.1	14548007	18543371	18875408	14285685	4885565
W01C9.4	4503301	18571478	1575000	111287	17105350
F11E6.5	18496985	17454617	10444345	13129088r	16151801
B0285.8	4557455	18603990	6671748	6978649	
Y49A3A.1	19527084	5174415	18580821	9910384	12841853
F23H11.9	10092647	12832369	12835319	12854793	
C01C10.3	15489111	7661996	19353717	12834446	11427110
F43H9.2	6685594	6755656	4758668	2137762	6691980
Y6B3B.10	137047	11641421	18490663	3264848	13385556
K09D9.2	117254	6166042	117218	10835506	19071838
K07C6.4	117219	2144294	117197	15147326	65684
K07C6.5	2134974	18088282	117225	1168128	117235
T04A8.16	6753258	7656959	4495099	2970661	2584822
F28H6.3	4757928	6318318	2143819	1083706	2144056
C06E7.3	13097429	5174529	19705457	284301	4557737
F13D12.6	2098347	4505989	13929457	12653639	3191969
F52B11.2	4557839	12833938	14776420	8393988	18594118
K03B8.3	6678862	285323	6981198	1083414	91080
C24A11.9	18575685	7657653	9507201	12848965	9966853
T09B4.8	13994255	13929196	2492864	1096025	12836724
T12A2.1	18579028	12836736	18490737	17511765	7705596
E01A2.i	8393446	6680019	4504011	9588455	556518
C46H11.2	18252634	3171877	4503759	19421731	2494584
M28.6	13507666	14249588			
C37H5.3	19527302	19353227	11545767	13649744	13385690
C15H9.7	16758776	2143818	12852371	4504937	12654129
K08A2.b	7446194				
B0280.3	6677767	15930194	1350838	18550866	17456433
C46E10.9	18561872	18595962	4505855	9910494	14250235
C47C12.3	11463867	14916471	6677645	6677643	6677647
T09F3.1	16551981	18590023			
ZK686.4	13385046				
Y116A8C.32	7513392	1620405	14318588	14165553	7513389
C09G9.7	4557827	4557823	110788	417447	280984
W02C12.3	15282044	4557755	1092105	6678884	13124344
F22A3.5	339895	18149007	107390	8567384	14782520
C02F4.2	4584820	19343933	8394030	14209665	6715568
F39D8.2	14780600	14043520	4758930	2190414	7710080

ZK757.3	11386878	11024680	18570004	6912352	13376275
D2089.2	12836671	19263985	16358983	12805349	7706043
F11A10.3	2327052	13111867	5729939	2687591	5931741
B0218.5	18201865	14781533	13122442	13435470	13097702
T05C12.1	20900385	18201865	20555151	6526520	13122442
Y53C12A.1	19264036	12746438	2914671	4758928	2460023
C16A11.3	110864	125290	4502885	4758008	13623342
ZK930.1	14728229	17028437	12836135	14150114	4507281
ZC504.4	3721838	18553056	3327188	6679060	18555837
M01B12.5	16549132	13899340	12856491	16163884	18152791
C06A1.3	130706	13994195	542987	4506003	1535
ZC302.1	5031921	11560107	5031923	9055282	1401337
C03D6.3	6755342	18042848	14750499	4506563	12833263
T19D2.2	4506283	17528929	4506285	1246236	17865337
C47D12.1	4507691	4151929	14748864	3694663	7512506
ZK909.3	12835118	18584663			
R107.4	7661946	9789983	7019547	12835969	9790253
C33H5.17	17939660	14017911	14249740	14042873	18204508
R07E5.1	14763089	13543933	13385692	8922283	12852170
C41D7.2	12853786	4557803	8134591	10945625	8099648
ZK675.1	4506247	6679519	1335864	6679517	4325111
F20H11.2	11990420	7023114	18255692	7662410	16163366
C44F1.5	12018268	729241	8176552	284522	423625
F46G11.3	12730586	4885251	17375734	13591947	12803719
K10D3.5	14042287	7019333	13435804	15079264	11360161
F41D9.1	14165549	19527318	11034851	7209313	5689447
F07C3.4	7657152		12856817	7305093	7705931
F45H7.4	9506973	8393959	16580805	3800869	4505811
C07E3.2	18550740	10434347	18598995	8480755	7512721
C38C10.1	107184	7669548	6678213	107185	8394408
C34C6.6	6679581	12084650	9663119	3005920	16197740
E02C12.3	17559196	17559194	17566398	17562326	17564240
F33G12.2	14150114	18044039	12838548	13385884	13542766
C32C4.1	16758912	4758622	13242172	15418950	5921784
C37A5.1	4759310	18044531	8923137	3335161	18476496
K04E7.2	2143888	2832268	1136776	4827008	15301458
C34G6.4	6755046	6755048	266517	2506118	307180
K05F1.6	4506999	8918937	2511670	4507005	15147378
ZK682.2	9719374	6912666	9719376	4885441	15487160
C13D9.7	18875376	13376449	17865509	17865499	12597441
F23F1.6	6671596	18181964	6981556	539952	1706187
F15H10.4	17505223	18426842	17473038	12005633	14767497
F59F5.1	7513431	5730045	6677997	4759116	4759112
C46F11.1	3355534	10092641	18564398	17390915	13569893
H27A22.1	12856921	18043218	6912618	2498823	12841414

T19B4.7	4885175	6681139	6978755	4505375	2078518
T27F7.1	6716764	7706353	12852884	12844196	12858406
C33A12.1	400650	4826848	14777313	12844560	6981260
F20D1.9	12845461	13375983	13899342	12837673	18848167
F14D12.2	17462167	10433878	18860509	13542844	14789889
W03C9.3	13027392	14746535	131797	13794267	6679599
F11A5.3	18088786	12837642	10946940	13929006	108107
R11A5.1	4501975	18201935	15929245	20909238	6753076
F53H8.1	9055268	6912240	19424296	12836141	10439979
T14D7.3	12851438	7949160	14736500	4507863	4200247
R01H2.3	4507157	8928391	8928392	7513715	7513686
T22D1.4	4506675	19527152	9857227	14124942	6981486
F54H5.3	8099350	12842294	14759532	4507867	7305623
K09B11.9	16359203	13431959	1171952	9507177	1171950
Y38E10A.c	8925888	8925890			
F49E12.4	4507789	10444495	8134778	4759282	18582039
F40H3.5	14336772	4826764	14494990	20899626	9957244
F49E11.4	13899332	18490353	6678423	14042040	15779065
M01E10.2	2065167	18201921	3182940	18201923	14757167
F26D11.10	16418341	6572227			
Y37D8A.1	9790141	5031597	2209347	18104938	13639437
Y17G7B.15	17434817	16945966	12697977	7661880	18551362
R107.6		14726914	7513045	16758540	12839687
C23F12.1	7677526	14744078	4557597	5419655	8885790
M106.5	11131728	13124696	1345668	4826659	19352984
C06G3.2	9910266	14424665	13194197	3891936	9910292
T28D6.2	135395	223556	135412	18601895	6755901
W09D10.3	4506673	11177148	12837801	17865556	12832973
W10D9.5	12842129	20902823			
F54D5.11	4504195	13386074	18043581	228300	11611843
F44B9.7	12847646	6677723	4506491	283966	13097123
Y71H10B.1	6912598	2668557	12841987	18544261	7706749
F21D5.5	14211141	6005836	5757919	12803393	14786918
F20D12.2	11225270	19923191	7513026	13938593	14780851
T22D1.10	5730023	6755382	4929561	9790083	4506753
C24A1.4	1263081	1585320	14286268	3005702	5730039
Y37A1A.1	3005702	5730039	1263081	14286268	1585320
C30F12.1	18604558	13376632	14750657	2224587	16041792
W09G3.1	18582801	7662058	18578718	6005796	14388374
F13E6.1	13385202	4507643	11125673	15723372	17450493
T21D12.3	9506989	16307140	5031957	11125356	10801584
B0041.5	17391272	12836420	10437002	13376774	4758846
B0286.4	11640600	7657385	12847218	7020899	6841484
D1054.14	20841107	20472100	20881439	14249602	14149989

F54C9.9	14602715	12804075	10438567	12711672	13649858
B0513.7	3005702	5730039	1263081	14286268	1585320
Y41D4A_3192.a	12052882	8922808	12849037	18548855	16550576
ZK686.3	14714487	6166601	1353701	6996934	14149775
B0041.3	12843216	14585867			
C05E11.1	14725463	12697975	16551959	15777193	15843561
F38A5.1	18559316	14714703	8922938	13529584	13899211
Y47G6A_245.b	11360017	5689555	14725071	11360104	18544047
R05F9.8	8099350	14759532	4507867	12842294	13928870
W04A4.5	14124974	16553697	18566093	15809018	14602672
Y49F6B.n	6919955	15278160	15928532	14767139	5901878
C47D12.7	13431657	6329805	8393672	6644293	18490684
C32D5.11	4504867	13645747	18255721	13905224	12585549
H32C10.3	9506623	4589536	14755049	6841238	3329427
T21C9.2	7023936	15297473	16518394	8980452	7705397
Y41E3.11	12803479	14786840	5901942	18204832	16041796
Y57G11C.17	14198202	8922621	4826524	14149736	18490309
T04C10.2	7019369	3894395	11177890	10433856	16923990
F13D11.1	8392842	19484058	130728	13111975	4557010
R04A9.4	124222	7546552	110568	4503535	6681293
C54H2.5	6755698	19557691	2414516	5531849	18571517

**Table XIII. Novel Genes that Reduce Fat Content And Viability/Growth**

F37E3.1	4505343	627397	15988381	5441937	12851646
Y37D8A.14	6680986	12858580	117100	18999392	4758038
Y57G11C.12	4505359	400384	13385492		
F28B3.1	17471893	6686133	14043353	17457389	6686101
C23H3.4	6678125	2853289	6685595	5454084	2136140
E04A4.7	229351	118007	118014	117994	117966
F01G10.1	12855432	388891	12018252	4507521	1729977
F44D12.4	18203658	16758060	5031715	9055336	18656362
F46E10.1	17390865	13376741	15559516	16553412	4164168
F57B9.2	4589658	12653967	16598275	19354215	7706214
H14A12.2	8393358	227665	68293	13543801	12832319
H15N14.2	20913355	13489067	6679140	134267	90219
K02F2..2	20892597	9951915	7709980	178277	13096485
K06A4.5	17921976	6912406	9910256	13637831	4433351
T05H4.4	1709233	1070443	4503327	19421846	553600
T05H4.5	127847	1070444	17943396	1709233	19745150
Y55F3A_750.e	12841560	6755911	16758644	4759274	7949156
B0285.1	14110390	14110387	7706549	4240297	14748750
C16C2.3	1352493	9966773	12836107	13249985	346209
W03F8.5	4504951	293690	126367	226290	6981142
W07E6.2	8922428	12804063	17390943	3043443	7305363
ZK1067.1	4503597	280818	119534	17432904	10880776

ZK675.1	4506247	6679519	1335864	6679517	4325111
C33D3.1	8648977	3123218	477102	15593990	5882288
C34H3.a	16506291	17298684	16877372	6754928	19344028
D1081.2	14719546	18655799	10048414	4507205	7546482
F10C1.5	13940223	18572015	11230443	12229781	11386173
F22A3.1	6912580	7305415	18204910	15214598	4557551
F23B12.7	1705659	5031625	6753402	10439934	10434534
F25H8.3	13626125	7242979	9910122	7243073	6685072
Y17G7A.2	18027804	11870006	18602376	6729087	19173808
C01F6.8	12848135	2197127	1095482	7513563	1060971
C56C10.8	17441804	2851417	115143	107909	29507
C56E6.1	11967969	16758590	14141178	16158955	15146444
W06D12.2	16306555	11560129	11177516	19483870	13124041
W10D9.5	12842129	12844929	9910382	17475714	16930809
Y61A9LA_75.a	7661980	18575034	14737076	18576250	7243183
C36B1.4	4506189	7106389	4092058	18577124	1346784
CD4.6	9910833	8394060	4506179	14768212	13543551
F23F12.6	2492517	5729991	1172637	3450955	7110701
F39H11.5	3914439	14198355	3915806	16165126	13928866
T23F2.1	12846285	14861836	9910440	12836608	18572937
Y38A8.2	4506197	11424309	6755202	8394082	17447021
C36E8.5	7106439	135490	5174735	14758306	12846758
D2024.6	12841166	5453597	16740716	1345694	6671672
F10C1.2	34228	125962	5031875	1072002	383110
F20G4.3	13928704	1346640	13431706	17978023	12667788
K07C5.1	5031571	15778930	14769120	12852068	1351867
T04C12.5	71621	4501885	16304154	1351867	16359158
Y19D2B.1	6678469	12850141	12839396	90217	2843123
ZK593.5	13259510	4139121	1419567	13259508	6681147
B0303.9	18105056	12621146	12859683	7514114	10439792
C02C6.1	6681207	729381	1083647	18093102	539580
C05D11.2	19343731	15553046	17978479	11345382	13385360
F29G9.3	12005732	4506957	12837633	4557471	5630084
F41C3.4	7634779	7705636	13385354	20340619	17488855
K02D10.5	16758654	6685966	12836691	12963651	4759154
T21E12.4	9506549	13384736	729378	18582791	2224591
B0035.7	631691	17455198	18545931r	1458139	18580602
B0035.8	223096	280961	18564726	4504263	15030326
B0035.9	12854993	12847763	4504301	70762	223582
B0041.4	2500343	11968086	16579885	12846949	1363989
C03C10.3	4557845	7106399	2500209	11256408	14743689
C04H5.6	14318701	14250712	14752410	4503293	7770157
C06A8.2	4507101	7243201			
C08B11.5	5032069	18582960	18582878	4504715	17489281
C09H10.2	4506651	14750580	13645150	9845295	17472837
C15F1.e	19424322	17511746	10436247	9966779	10433787
C15H11.9	3183219	14719402	10946930	12857046	12845436
C16A3.3	12834845	2498864	17454886	4454542	13385288
C16A3.4	15529978	8922413	10435029	18583383	12698069

C16A3.6	14042167	14210516	14747081	15341814	12853682
C26D10.1	4502801	14278207	4389390	87057	132171
C26F1.9	18592185	18590969	4506647	14738021	17449824
C27F2.4	12846835	16226067	13384748	12843473	12652833
C29F5.3	7662204	4521188	14750657	2224587	16041792
C37H5.8	16158324	12653415	6754256	1072476	4758570
C42D4.8	5902062	133327	2145091	7434727	4505939
C47D12.6	14861852	12653491	14714853	12845562	4507367
C50F4.5	223096	280961	18564726	15030326	16306566
C52E4.3	4759158	17471847	2833357	12862083	7657315
D100T.6	14195014	14782930	13540714	13639605	14195007
F09E8.3	4505253	3108220	5725250	12655021	3986757
F09F7.3	4505941	7022241	8922399	6677789	3005758
F18A1.5	4506583	18390321	12860240	2624702	13096131
F20D12.4	15214617	4759344	11387254	18546147	126369
F22B3.1	12854993	12847763	4504301	70762	223582
F22B5.2	8393308	6492222	2460200	4503517	14280325
F22B5.9	15296128	5032011	6841566	12845588	12644592
F26F4.10	586063	18043638	15149476	12847471	1711647
F26F4.11	14589953	1710659	4406232	476961	6680928
F32E10.4	6680598	4504901	6680596	14758897	2654139
F37C12.11	4506699	17390310	12841661	13592073	12964241
F37C12.9	5032051	12083607	10181112	7440317	16158168
F45E12.3	11140811	13270467	13259127	16307345	13386300
F45F2.13	4504281	4504299	386772	18595043	70749
F54E12.1	4504281	4504299	386772	18595043	70749
F54E12.5	631691	17455198	121983	8922758	18545931
F55C5.8	7657617	134889	7513444	18044248	14041927
F55F10.2	7529573	17512348	4678973	15029526	18604727
F55G1.10	631691	17455198	18545931	1458139	18580602
F58A4.4	6679459	110830	3676248	4506051	12847590
H02I12.7	631691	17455198	18545931	1458139	18580602
H06H21.3	4758254	12859663	11418342	4503499	3746340
H06I04.i	18028291	17017991	13384672	15126717	7019917
H19M22.1	18676514	18702313	7959295	9507013	18860896
H23L24.c	11024694	17480509	18587523	18572403	12853018
K03A1.1	7305139	70743	70749	4504281	2119013
K12D12.2	18566874	1504030	12852259	17646641	
R05D11.3	5031985	2914436	2780953	7246005	17451119
T01C3.6	4506691	18549572	18591367	7305445	70920
T02G5.9	16716381	586059	11095909	505108	2501023
T10C6.11	223096	280961	18564726	87672	4504263
T10C6.12	631691	17455198	121983	8922758	18545931
T10C6.13	4504281	4504299	386772	18595043	70749
T13H5.4	1082801	18202846	5803167	12854243	18578493
T23B12.2	14756630	12832465	12653925	7705722	9956063
T28F3.2	18565400	13242237	5729877	1708309	347019
VW02B12L.1	12643966	12025532	7329154	13928826	3915315
W07E6.1	189422	5453792	14784289	477430	12653741

Y106G6H.3	4506631	17463853	17435581	18561308	18087841
Y41D4A_3457.d	4758844	6093462	18875386	16758834	18559983
Y47D3A.c	6679409	6015013	15858951	118838	479803
Y62E10A.d	18561782	133063	12849327	1173072	4506671
Y71G12A_187.b	5901998	4507131	14755615	4507133	13644938
Y76B12C_66.c	18570089	1706101	16878041	16751835	9558725
ZK550.4	11432489	5031727	12847734		
ZK652.1	14755615	4507131	5901998	4507133	11138539
C06A1.1	2144498	17865351	6005942	6678559	1174636
C15H9.4	14916851	14753980	14916847	14724805	13874437
C30B5.6	12834595	14250466	18605027	7706047	12856705
C30C11.2	15310156	16550621	4506229	12652653	15126760
C37H5.5	18482381	18389431	10434534	7208452	10439934
D1054.3	12846547	12841721	12861014	5730041	18567662
F19F10.9	2342526	8394236	10863889	4427065	13928810
F32E10.1	13477303	13430872	17437341	10436236	14010904
F33A8.1	10047283	10438214	13385386	10439972	14727768
F54F2.7	8923431	17470330	7513001	13446227	14010849
R12E2.2	7705322				
R144.2	13431763	4240137	7706224	13435542	14603356
Y51H4A.m	8923726	4153862	18568744	7021918	
Y53C12B.2	17390336	10047140	13384846	12844974	
ZK121.c	7662442	7661874	15149484	18599919	11360196
ZK546.2	12834355	18597991	12833443	12859847	12849514
ZK795.3	12835200	12850634	18478512	15529982	12845999
C04G2.6	18582324	19923416	7451876	7674415	7512650
F41H10.7	17454617	20137972	18496985	12836437	16151801
T10B5.5	6671704	5453607	12848801	1800303	13540473

### Novel Genes that Increase Fat Content

Table XIV shows *C. elegans* genes and their mammalian orthologs that have not previously been shown to increase fat content when inactivated. This list identifies the *C. elegans* genes by *C. elegans* cosmid name and open reading frame number. The mammalian orthologs are listed by the Genbank protein accession number.

**Table XIV. Novel Genes that Increase Fat Content**

C33A12.6	549160	6537138	19527110	5803213	18308170
VF13D12L.1	7022512	7705558	11493904	12963757	11281329
C37F5.1	7767065	4100456	535923	14758312	4885201
C56C10.10	4502009	1765936	7709982	6225016	8248030
F16B4.9	6681852	12803755	5453940	346286	1144348
K10C3.6	7657395	6681852	6680239	11559939	227511
R11H6.5	20534593	4758602	13385872	6855637	13278459
C04G2.2	20900385	20555151	18201865	7949025	20149530
C09G5.8	4589654	20888031	9965252	9966409	9965248
C18H9.7	20839618	6677669	15619013	20178328	631056
C24F3.2	6005956	11560052	12963553	12835696	13435759
F39B1.1	11259849	20843206	20561002	4505799	6755058
F46C5.6	10047321	17402886	12858102	1702997	11386167
F56D5.9	1695739	2642034	2143944	7513459	13647589
F56H11.6	18201865	14781533	5579454	547767	11545751
R10D12.10	18201865	14781533	1311054	1346368	20149530
T04B2.2	6679773	1673620	6003683	4885231	4503687
T04C9.1	7662208	6433901	14587851	13386454	20340540
W03A5.4	19923689	19923274	7514059	1857137	1857139
W08D2.1	17402916	5020354	16716413	17402914	13518017
Y11D7A.9	7657102	6606290	7512548	14602865	16758956
ZC513.1	14583090	18377358	2497615	13929458	5453914
C43H6.9	3935134	2598978	1169965	4504119	3287976
F08H9.5	20903753	6492289	4557503	20473663	14388673
F32B6.9	4759310	8923137	18044531	3335161	18476496
ZC410.4	9988112	14149764	18652258	11496265	4504851
C18H9.5	9719374	6912666	9719376	18252796	16758166
F14E5.1	687622	8394301	121750	20301952	6755550
F52H2.2	4507053	6103627	3970791	7106415	6319236
C04G2.4	13928870	7305623	6671046	8099350	20070156
F32B6.6	8099350	14759532	3320446	12842294	13928870
C15A11.3	19070657	11907926	11934950	11907928	11934951
Y38F1A.9	14211895	14748249	3928489	7513113	7657361
K02D7.5	6677733	12852065	20872938	13543580	10047124
C14A4.1	19527182	13775228	20862789	3482908	16306483
C44E4.5	14042905	12654293	14718862	14745963	20902053
D1007.5	20892497	8922756	20845045	10435222	13385900
F25H8.1	18381001	14724179	4454968	18572769	18043204
F26H9.4	20893587	20536806	14029540	3413920	12620200
W06H12.1	13236593	20454983	20521730	16307459	20864376
Y57A10B.1	12836671	19584503	8923613	7513036	19263985

### **Novel Genes with No Previously Identified Function**

Below are shown lists of *C. elegans* genes and mammalian polypeptides for which no known function has been previously identified. This list identifies the *C. elegans* genes by *C. elegans* cosmid name and open reading frame number. The mammalian orthologs are listed by 5 the Genbank protein accession number. It has been demonstrated herein that the genes and polypeptides identified in Tables XV, XVI, and XVII are involved in fat metabolism.

**Table XV Reduced Fat: No Previous Function Identified**

<b><i>C. elegans</i> Gene</b>	<b>Mammalian Accession Numbers</b>			
AH10.1	13376741	15559516	17390865	
C06E7.3	13097429			
F23H11.9	10092647			
T12A2.1	18579028	18490737		
Y41E3.10	12653785			
C46E10.9	18561872	18595962		
T09F3.1	16551981	18590023	18546120	
C33H5.17	17939660	14249740	14042873	18204508
F41D9.1	14165549	11034851		
K10D3.5	14042287	15079264	11360161	
M01B12.5	16549132	13899340		
R07E5.1	14763089	8922283		
ZK909.3	18584663			
F33G12.2	14150114			
F14D12.2	17462167	10433878	14789889	
F20D1.9	13375983	18848167		
F49E11.4	13899332	18490353	14042040	
B0286.4	7020899	6841484		
B0513.7	3005702			
C05E11.1	16551959			
C30F12.1	13376632	16041792		
F38A5.1	18559316	14714703	8922938	13529584
F54C9.9	14602715	12804075	10438567	12711672
Y41D4A_3192.a	12052882	8922808	18548855	16550576
H32C10.3	9506623			
T21C9.2	7023936			

**Table XVI Reduced Fat and Reduced Growth/Viability: No Previous Function Identified**

<b>C. elegans Gene</b>	<b>Mammalian Accession Numbers</b>				
F46E10.1	17390865	13376741	15559516	16553412	4164168
Y53C12B.2	17390336	10047140	13384846	12844974	
F32E10.1	13477303	13430872	17437341r	10436236d	14010904
F08D12.7	19527308	4557445			
C53B7.4	15929392				
W07E6.2	8922428	12804063	17390943	3043443	
Y17G7A.2	18027804				
C15H11.9	3183219				
C16A3.4	15529978	8922413	10435029		
C16A3.6	14042167				
C26F1.9	18592185	18590969			
C47D12.6	14861852	14714853			
H06I04.i	18028291	7019917			
Y62E10A.d	18561782				
C15H9.4	14916851	14916847			
F54F2.7	8923431				
Y51H4A.m	8923726	18568744	7021918		

**Table XVII Increased Fat Genes: No Previous Function Identified**

<b>C. elegans Genes</b>	<b>Mammalian Accession Numbers</b>				
VF13D12L.1	7022512	11281329			
C44E4.5	14042905	12654293	14745963		
F25H8.1	18381001	14724179	4454968	18572769	
W06H12.1	13236593				

5

### Working Examples of Mammalian Orthologs

#### *LPO-3 Mammalian Ortholog*

The ABC transporter, *lpo-3*, encodes the Wormpep database protein C34G6.4 (Wormpep is the database of translated and assembled open reading frames derived from the *C. elegans* genome sequence). RNAi analysis of *lpo-3* indicated that its inactivation dramatically reduces fat levels in wild-type nematodes, and in high fat nematode mutants (e.g., *tub-1*, *tph-1*, *daf-2*, *lpo-1*, and *lpo-6*). The inactivation of C34G6.4 reverses a *C. elegans* high fat phenotype, regardless of whether the increase in fat level results from a neural defect, such as a defect in *tub-1*, which is expressed in sensory neurons, or a defect in *tph-1*, which is expressed in serotonergic neurons; or from a neuroendocrine defect, such as a defect in the *daf-2* insulin receptor gene, which may be expressed in neurons (Wolkow et al., *Science* 290:147-50, 2000).

Blast analysis of C34G6.4 was carried out versus the Genbank protein database, that contains the complete genome sequences of humans, *Drosophila*, and *C. elegans* (as well as yeast and other microbes). This analysis identified C34G6.4 highly related proteins. These results are shown below.

```

5      gi|17508505|ref|NP_491707.1| (NM_059306) ABC transporter [C. 2131 0.0 ... (C34G6.4)
gi|7511698|pir||T31073 multidrug resistance p-glycoprotein ... 1539 0.0 ... (mouse)
gi|6755046|ref|NP_035205.1| (NM_011105) ATP-binding cassette... 969 0.0
10     gi|2506118|sp|P08183|MDR1_HUMAN Multidrug resistance protein... 964 0.0 (human shown below)
gi|2506118|sp|P08183|MDR1_HUMAN Multidrug resistance protein 1 (P-glycoprotein 1)
gi|1070659|pir||DVH1 multidrug resistance protein 1 - human
gi|386862|gb|AAA59576.1| (M29447) P glycoprotein [Homo sapiens] Length = 1280

Score = 964 bits (3277), Expect = 0.0
15     Identities = 573/1256 (45%), Positives = 828/1256 (65%), Gaps = 18/1256 (1%)

Query: 13      KPLLKRSHSDDSSIDEVKLTNYGIFYYTQGVDLLLLITGTVAAVIHGAGFPLLAIVLG 72
K   K ++ S+    E    ++ + + F Y+ +D L ++ GT+AA+IHGAG PL+ +V G
Sbjct: 14      KNFFKLNKSEKDKEKKPTVSFSMFRYSNWLDKLYMVVTAAIIHGAGLPLMMLVFG 73
20     Query: 73      GMTTVFLRAQN-SDFVVGVVDNVNPEGLVPISLDEFNSEVVKYCIYYLVLGVLMFTSYVQ 131
MT +F A N D + + N +    ++    ++ +Y YY +G +    +Y+Q
Sbjct: 74      EMTDIFANAGNLEDLMSNITNRSDINDTGFPMN-LEEDMTRYAYYSIGAGVLVAAYIQ 132

25     Query: 132     IACFESYAERLVHKLRQNYLKAILRQQIQWFDKQQTGNLTARLTDLERVREGLGDKFAL 191
++ + A R +HK+R+ + AI+RQ+I WFD G L RLTDD+ ++ EG+GDK +
Sbjct: 133     VSFWCCLAAGRQIHKIRKQFFHAIRMRQEIGWFDVHDVGELNTRLDDVSKINEGIGDKIGM 192

30     Query: 192     LVQMFAAFLAGYGVGFYSWSMTLVMMGFAPLIVLSGAKMSKSMATRTRVEQETYAVAGA 251
Q A F G+ VGF W +TLV++ +P++ LS A +K +++ T E YA AGA
Sbjct: 193     FFQSMATFFTGFIVGFTRGWKLTLVILAISPVLGLSAAWAKILSSFTDKELLAYAKAGA 252

35     Query: 252     IAEETFSSIRTVHSLNGHKRELDRFYNALEVGRQTGIVKYCYMGIGVGFSNLCKMYSSYAL 311
+AEE ++IRTV + G K+EL+R+ LE ++ GI K I +G + L +Y+SYAL
Sbjct: 253     VAEEVLAIRTVIAFGGQKKELEYRNKLEEAKRIGIKKAITANISIGAAFLLIYASYAL 312

40     Query: 312     AFWYGSTLIINDPTFDRGLIFTVFFAVLSGSTLGGALPHLASFGTARGAASTVLRVINS 371
AFWG+TL+++ + G + TVFF+VL G+ S+G A P + +F ARGAA + ++I++
Sbjct: 313     AFWYGTTLVLSGE-YSIGQVLTFFSVLIGAFSVGQASPSIEAFANARGAAYEIFKIIDN 371

45     Query: 372     HPKIDPYSLEGILVDNMKGDISFKDVHFRYPSRKDIHVKGISLELKAGDKIALVGSSGC 431
P ID YS G DN+KG++ F++VHF YPSRK++ +LKG++L++++G +ALVG+SGC
Sbjct: 372     KPSIDSYSKSGHKPDNIKGNLFPNVHPSYPSRKEVKILKGLNLKVQSGQTVAI.VGNSGC 431

50     Query: 432     GKSTIVNLLQRFYDPTKGRVLIDGVDLREVNVHSLREQIGIVSQEPVLFDTIYENIKMG 491
GKST V L+QR YDPT+G V +DG D+R +NV LRE IG+VSQEPVLF TI ENI+ G
Sbjct: 432     GKSTTVQLMQRLYDPTEGMVSVDGQDIRTINVRFLREIIGVVSQEPVLFATTIAENIRYQ 491

55     Query: 492     NEHATHDQVVEACKMANANDFIKRLPDGYGTRVGEKGVLQLSGGQKQRIAARIARALVKNPKI 551
E+ T D++ +A K ANA DFI +LP + T VGE+G QLSGGQKQRIAARIARALV+NPKI
Sbjct: 492     RENVTMDEIEKAVKEANAYDFIMKLPHKFDLTVGERGAQLSGGQKQRIAARIARALVRNPKI 551

Query: 552     LLLDEATSALDTEAEREVQGALDQAQAGRTTIIVAHRLSTIRNVDRIFVFKAGNIVESGS 611
LLLDEATSALDTE+E VQ ALD+A+ GRTTI++AHRLST+RN D I F G IVE G+
Sbjct: 552     LLLDEATSALDTESEAVVQVALDKARKGRTTIVIAHRLSTVRNADVIAGFDDGVIVEKGN 611

Query: 612     HEELMSKQGIFYDMTQAQVVRQQ-----QQEAGKDIEDTISESAHSHLSRKSSTRSA 663
H+ELM ++GI++ + Q +        + ++ D + S + S L RK STR +

```

Sbjct: 612 HDELMKEKGIFYKLVTMQTAGNEVELENAADESKSEIDALEMSSNDSRSSLIRKRSTRRS 671  
 Query: 664 I--SIATSIHQLAEEVEECKAPPTSMFKIFKFNGDKVGWFIGGIFGAFIFGSVTPVFALV 721  
 + S A +E + PP S ++I K N + +F+ G+F A I G + P FA++  
 5 Sbjct: 672 VRGSQAQDRKLSTKEALDESIPPVSFWRIMKLNLTTEWPYFVVGVFCAIINGLQPAFAII 731  
 Query: 722 YAEIFNVYSLPAD--QMQANVYFWCGMFVLMGITFFVGFFTSANCLGRCGESLTMKLRFE 779  
 ++++I V++ D + N + +F+ +GI F+ FF G+ GE LT +LR+  
 Sbjct: 732 FSKIIGVFTRIDDPETKRQNSNLFSLLFLALGIISFITFFLQGFTFGKAGEILTKRLRYM 791  
 10 Query: 780 AFKNLLRQDIAFYDDLHRGTGKLCTRPATDAPNVR-YVFTRLPVVLASIVTICGALGIGF 838  
 F+++LRQD++++DD ++ TG L TR A DA V+ + +RL V+ + I + + I F  
 Sbjct: 792 VFRSMLRQDVFSWDDPKNTTGALTTRLANDAAQVKGAIGSRLAVITQNIANLGTGIIISF 851  
 15 Query: 839 YYGWQLALILVVMPPLLVMGGYFEMQMRFGKQIRDQLLEEAGKVASQAVEHIRTVHSN 898  
 YGWQL L+L+ +VP++ + G EM+M G+ ++D + LE AGK+A++A+E+ RTV SL  
 Sbjct: 852 IYGWQLTLLLIAIVPIIAIAGVVEMKMLSGQALKDKKELEGAGKIATEGRVVSLT 911  
 20 Query: 899 RQEKFHFTYCEYLREPFTNLKHAHTYGAFFVAFAPSQSLIFFMYAAFYLGSI FVNQQAMQP 958  
 ++++F Y + L+ P+ +L+ AH +G F+F+Q++++F YA F G+ V + M  
 Sbjct: 912 QEOKFEHMYAQSLQVPRNLSLRKAHIFGITFSFTQAMMYFSYAGCFRFGAYLVAHKLMSF 971  
 Query: 959 IDVYRVFFAISFCGQMIGNNTSFIPDVVKARLAASLLFYLIEHPTPIDSDSGIV-KPI 1017  
 DV VF A+ F +G +SF PD KA++A+ + +IE IDS S G++ +  
 25 Sbjct: 972 EDVLLVFSAVVFGAMAVGQVSSFAPDYAKAKISAHHIIEKTPLIDSSTEGLMPNTL 1031  
 Query: 1018 TGNISIRNVFFNYPTRKDTKVLQGFTLDIKAGKTVLGHSGCGKSTIMGLLERFYNQDK 1077  
 GN++ V FNYPTR D VLQG +L++K G+T+ALVG SGCGKST++ LLERFY+  
 30 Sbjct: 1032 EGNVTFGEVVFNYPTRPDIPVLPQGLSLEVKKGQTLALVGSSGCGKSTVVQLLERFYDPLA 1091  
 Query: 1078 GMIMIDGDNIRNLNISSLREQVCIVSQEPTLFDCТИGENICYGTN-RNVTYQEIVEAAKM 1136  
 G +++DG I+ LN+ LR + IVSQEP LFDC+I ENI YG N R V+ +EIV AAK  
 Sbjct: 1092 GKVLLDGKEIKRLNVQWLRAHLGIVSQEPILFDCSIENIAYGDNRSRVSQEEIVRAAKE 1151  
 35 Query: 1137 ANIHNFILGLPDGYDTHVGEKGTQLSGGQKQRIAIARALVRSPSVLLDEATSALDTESE 1196  
 ANIH FI LP+ Y T VG+KGTQLSGGQKQRIAIARALVR P +LLDEATSALDTESE  
 Sbjct: 1152 ANIHAFIESLPNKYSTKVGDKGTQLSGGQKQRIAIARALVRQPHILLDEATSALDTESE 1211  
 40 Query: 1197 KIVQEALDAAKQGRTCLVIAHRLSTIQNSDVI AIVSEKGKIVEKGTHDELIRKSEIY 1252  
 K+VQEALD A++GRTC+VIAHRLSTIQN+D+I + G++ E GTH +L+ + IY  
 Sbjct: 1212 KVVQEALDKAREGRTCIVIAHRLSTIQNADLIVVFQNGRVKEHGTHQQLLAQKGIY 1267  
 Score = 379 bits (1280), Expect = e-103  
 Identities = 238/622 (38%), Positives = 367/622 (58%), Gaps = 25/622 (4%)  
 45 Query: 18 RSHSSDSSIDEVKLTNYGIFYYTQGVDLILLITGTVAAVIHGAGFPLLAIVLGGMTTV 77  
 R S+ ++DES ++ + I + + G A+I+G P AI+ + V  
 Sbjct: 680 RKLSTKEALDESIPPVSFWRIMKLNLTTEWPYFVVGVFCAIINGLQPAFAIIFSKIIGV 738  
 50 Query: 78 FLRAQNSDFVVGVDNVNP EGLVPISLDEFNSEVVKYCIYYLVGVLMFFTSYVQIACFES 137  
 F R + + N+ + + +L LG++ F T ++Q F  
 Sbjct: 739 FTRIDDPETKRQNSNL-----FSLLFLALGIISFITFFLQGFTFGK 779  
 55 Query: 138 YAERLVHKLRQNYLKAILRQQIQWFD--KQQTGNLTARLTDDLERVREGLGDKFALLVQM 195  
 E L +LR + + +LRO + WFD K TG LT RL +D +V+ +G + A++ Q  
 Sbjct: 780 AGEILTKRLRYMVFRSMLRQDVFSWDDPKNTTGALTTRLANDAAQVKGAIGSRLAVITQN 839  
 Query: 196 FAAFLAGYGVGFFYSWSMTLVMMGFAPLIVLSGAKMSKSMATRTRVEQETYAVAGAIAEE 255  
 A G + F Y W +TL++ P+I ++G K ++ + + + AG IA E  
 60 Sbjct: 840 IANLGTGIIISFIYGWQLTLLLIAIVPIIAIAGVVEMKMLSGQALKDKKELEGAGKIA 899  
 Query: 256 TFSSIRTvhslngkrelDRFynalevgrqtgivkyycymgigvgfsnLCMyssyalafwy 315



```

Query: 1130 IVEAAKMANIHNFILGLPDGYDTHVGEKGTQLSGGQKQRIAIARALVRSPSVLLLDEATS 1189
       I +A K AN ++FI+ LP +DT VGE+G QLSGGQKQRIAIARALVR+P +LLLDEATS
Sbjct: 500 IEKAVKEANAYDFIMKLPHKFDTLVGERAQLSGGQKQRIAIARALVRNPKILLLDEATS 559

5   Query: 1190 ALDTESEKIVQEALDAAKQGRCLVIAHRLSTIQNSDVIAIVSEGKIVEKGTHDELIRKS 1249
       ALDTESE +VQ ALD A++GRT +VIAHRLST++N+DVIA +G IVEKG HDEL+++
Sbjct: 560 ALDTESEAVVQVALDKARKGRTTIVIAHRLSTVRNADVIAGFDDGVIVEKGHNDELMKEK 619

10  Query: 1250 EIYQKFCETQ 1259
     IY K Q
Sbjct: 620 GIYFKLVTMQ 629

```

The following exemplary BLAST search illustrates how the searches were carried out and how the list of genes was annotated. The C34G6.4 blast analysis also identified the 15 following related proteins.

gi 19743730 gb AAL92458.1	(AY082609) ATP-binding cassette ...	963	0.0
gi 307180 gb AAA59575.1	(M14758) P-glycoprotein [Homo sapi...]	961	0.0
gi 8926217 gb AAF81747.1	(AF269224) his-tagged-multidrug r...	959	0.0
gi 266517 sp P21447 MDR3 MOUSE	Multidrug resistance protein...	958	0.0
gi 6755048 ref NP_035206.1	(NM_011076) Abcb1a; P glycoprot...	958	0.0
gi 126928 sp P21449 MDR2 CRIGR	Multidrug resistance protein...	957	0.0
gi 4505769 ref NP_000918.1	(NM_000927) ATP-binding casset...	957	0.0
gi 2149087 gb AAB58489.1	(U78609) multidrug resistance pro...	955	0.0
gi 2852441 gb AAC02113.1	(AF045016) multidrug resistance p...	954	0.0
gi 126924 sp P21448 MDR1 CRIGR	Multidrug resistance protein...	952	0.0
gi 833699 gb AAA75000.1	(U17608) multidrug resistance prot...	952	0.0
gi 7739773 gb AAF69007.1 AF257746_1	(AF257746) multidrug re...	952	0.0
gi 1362689 pir  S55692	multidrug resistance protein homolog...	952	0.0

When the most closely related human amino acid sequence (LOCUS: MDR1\_HUMAN 1280 aa linear PRI 16-OCT-2001; DEFINITION: Multidrug resistance protein 1 (P-glycoprotein 1). ACCESSION P08183) was used as a query versus the Genbank wormpep database, the 35 C34G6.4 transporter had the second highest homology score. (The top four homologs have nearly equivalent scores of about 1000 bits).

Sequences producing significant alignments:			(bits)	Value
40 gi 17558664 ref NP_507487.1	(NM_075086) multidrug resistan...	1081	0.0	
gi 17508505 ref NP_491707.1	(NM_059306) abc transporter [C...	1073	0.0	
gi 17541710 ref NP_502413.1	(NM_070012) multidrug resistan...	1022	0.0	
gi 462583 sp P34712 MDR1 CAEEL	Multidrug resistance protein...	1021	0.0	
45 gi 17569145 ref NP_509902.1	(NM_077501) p-glycoprotein [Ca...	902	0.0	
gi 17569143 ref NP_509901.1	(NM_077500) multidrug resistan...	902	0.0	
gi 462584 sp P34713 MDR3 CAEEL	Multidrug resistance protein...	879	0.0	
gi 17567265 ref NP_510126.1	(NM_077725) p-glycoprotein (MD...	824	0.0	
gi 17567269 ref NP_510128.1	(NM_077727) p-glycoprotein (MD...	820	0.0	
50 gi 17567267 ref NP_510127.1	(NM_077726) p-glycoprotein (MD...	766	0.0	
gi 17569909 ref NP_509812.1	(NM_077411) p-glycoprotein [Ca...	752	0.0	
gi 17569907 ref NP_509813.1	(NM_077412) p-glycoprotein [Ca...	735	0.0	
gi 17550138 ref NP_509810.1	(NM_077409) p-glycoprotein [Ca...	704	0.0	
gi 17569911 ref NP_509811.1	(NM_077410) p-glycoprotein [Ca...	697	0.0	

5	gi 7508148 pir  T25082	hypothetical protein T21E8.2 - Caeno...	622	e-178
	gi 7508147 pir  T25083	hypothetical protein T21E8.1 - Caeno...	601	e-172
	gi 17567271 ref NP_510129.1	(NM_077728) p-glycoprotein (MD...	536	e-152
	gi 17532731 ref NP_495674.1	(NM_063273) multidrug resistant...	351	2e-96
	gi 17543740 ref NP_502776.1	(NM_070375) ABC transporter [C...	347	3e-95
	gi 17511077 ref NP_491754.1	(NM_059353) transporter protein...	339	7e-93
	gi 7511165 pir  T32865	hypothetical protein ZK484.2 - Caeno...	339	8e-93
	gi 7510080 pir  T31617	hypothetical protein Y50E8A.m - Caen...	331	2e-90

- 10 Interestingly, MDR1 has been implicated in mammalian lipid transport (van Helvoort et al., *Cell* 87:507-17, 1996). (MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine (van Helvoort A. et al., *Cell* 87:507-17, 1996). The ABC transporters are promising drug targets because their natural targets are small molecules, which represent good candidates for drug development.
- 15 Drugs that inhibit ABC transporter activity, particularly with respect to the transport of fats and fat metabolites, could be identified using the methods of the invention.

#### *F11E6.5 Mammalian Ortholog*

Another RNAi clone that caused a reduced fat phenotype in wild-type nematodes, as well 20 as in the increased fat mutant nematodes, e.g., *tub-1*, *tph-1*, *daf-2*, *lpo-1*, and *lpo-6*, was F11E6.5. F11E6.5 encodes a transmembrane protein that functions to elongate fatty acids. Epistasis analysis indicates that F11E6.5 acts at the same point in the pathway as the ABC transporter gene, C34G6.4. Blast analysis of F11E6.5 identified the following highly related proteins.

25	gi 17539766 ref NP_503114.1	(NM_070713) GNS1/SUR4 family [ .496	e-139	....it self
	gi 8489829 gb AAF75771.1 AF265296_1	(AF265296) putative mul.166	2e-40	...drosophila noa gene
	gi 7294075 gb AAF49430.1	(AE003526) Baldspot gene product 166	2e-40	drosophila bald spot gene...same as noa above
30	gi 17540336 ref NP_500793.1	(NM_068392) integral membrane ...	160	1e-38
	gi 17540774 ref NP_501689.1	(NM_069288) Yeast YJT6 like [C...	158	5e-38
	gi 17540338 ref NP_500797.1	(NM_068396) F41H10.8.p [Caenor...	158	6e-38
	gi 17539514 ref NP_501147.1	(NM_068746) D2024.3.p [Caenorh...	147	8e-35
35	gi 17454617 ref XP_058360.1	(XM_058360) similar to CIG30 [...	146	2e-34
	gi 18496985 ref NP_569717.1	(NM_130450) long chain fatty a...	145	3e-34
	gi 20137972 sp Q9HB03 ELO3_HUMAN	Elongation of very long ch...	145	5e-34
	gi 13129088 ref NP_076995.1	(NM_024090) long-chain fatty-a...	143	2e-33
	gi 11875973 emb CAC18863.1	(AL499614) transmembrane protei...	135	5e-31
40	gi 16151801 dbj BAB69888.1	(AB071986) fatty acid elongase ...	131	7e-30

F11E6.5 blast analysis identified the most closely related human homologue as “Elongation of very long chain fatty acids protein 3 (Accession number: Q9HB03).” The human homolog is an

integral membrane protein that is likely found in the endoplasmic reticulum where it has been implicated in the synthesis of very long chain fatty acids and sphingolipids; it may catalyze one or both of the reduction reactions required for fatty acid elongation, i.e., conversion of beta-ketoacyl CoA to beta-hydroxyacyl CoA or reduction of trans-2-enoyl CoA to the saturated acyl

5 CoA derivative.

When this human homologue was blasted versus the *C. elegans* database, F11E6.5 was identified as the second most closely related protein ( $e^{-35}$ ). The results of the blast analysis are shown below.

	Sequences producing significant alignments:	(bits)	Value
gi 17539514 ref NP_501147.1	(NM_068746) D2024.3.p [Caenorh...]	<u>164</u>	3e-41
gi 17539766 ref NP_503114.1	(NM_070713) GNS1/SUR4 family [...]	<u>143</u>	8e-35
gi 17552588 ref NP_499056.1	(NM_066655) Yeast hypothetical...	<u>141</u>	3e-34
gi 17537431 ref NP_497086.1	(NM_064685) Y53F4B.2.p [Caenor...]	<u>134</u>	5e-32
gi 17540338 ref NP_500797.1	(NM_068396) F41H10.8.p [Caenor...]	<u>129</u>	2e-30
gi 17540774 ref NP_501689.1	(NM_069288) Yeast YJT6 like [C...]	<u>125</u>	2e-29
gi 17540336 ref NP_500793.1	(NM_068392) integral membrane ...	<u>113</u>	7e-26
gi 17540772 ref NP_501691.1	(NM_069290) Yeast GNS1 like [C...]	<u>101</u>	3e-22

20

### T14E8.3 Mammalian Ortholog

T14E8.3 encodes a G protein coupled receptor. T14E8.3 RNAi caused a reduced fat phenotype in wild-type nematodes, and in *tub-1*, *tph-1*, *daf-2*, *lpo-1*, and *lpo-6* mutant 25 nematodes. Blast analysis of the T14E8.3 amino acid sequence indicated that the most closely related mammalian proteins are dopamine D2 receptors.

	Sequences producing significant alignments:	(bits)	Value
gi 17569809 ref NP_509106.1	(NM_076705) G-protein coupled ...	<u>1982</u>	0.0
gi 17562444 ref NP_505478.1	(NM_073077) dopamine receptor ...	<u>133</u>	1e-29
gi 1363347 pir  D56849	dopamine receptor-like protein D222 ...	<u>130</u>	6e-29
gi 7381416 gb AAF61479.1 AF176812_1	(AF176812) dopamine rec...	<u>130</u>	1e-28 (Human)
gi 17986270 ref NP_057658.2	(NM_016574) dopamine receptor ...	<u>130</u>	1e-28
gi 3820492 gb AAC78779.1	(AF050737) dopamine D2 receptor [...]	<u>129</u>	1e-28
gi 4503385 ref NP_000786.1	(NM_000795) dopamine receptor D...	<u>129</u>	1e-28
gi 405310 gb AAB26819.1	(S62137) D2 dopamine receptor [Hom...]	<u>129</u>	1e-28
gi 11344838 gb AAG34495.1	(AF293962) dopamine D2 receptor ...	<u>129</u>	2e-28
gi 1706283 sp P52702 D2DR_CERAE	D(2) dopamine receptor >gi ...	<u>129</u>	2e-28
gi 203906 gb AAA41075.1	(M36831) dopamine receptor subtype...	<u>129</u>	2e-28
gi 11344842 gb AAG34497.1	(AF293964) dopamine D2 receptor ...	<u>129</u>	2e-28
gi 1706284 sp P53453 D2DR_FUGRU	D(2)-LIKE DOPAMINE RECEPTOR...	<u>129</u>	2e-28
gi 6753680 ref NP_034207.1	(NM_010077) dopamine receptor 2...	<u>129</u>	2e-28
gi 11344837 gb AAG34494.1	(AF293962) dopamine D2 receptor ...	<u>129</u>	2e-28
gi 226700 prf  1603358B	D2 dopamine receptor 2in [Rattus no...]	<u>128</u>	3e-28
gi 1363346 pir  C56849	dopamine receptor-like protein D215 ...	<u>128</u>	4e-28
gi 226699 prf  1603358A	D2 dopamine receptor 2in [Bos tauru...]	<u>127</u>	7e-28

5	<u>gi 118205 sp P20288 D2DR_BOVIN</u>	D(2) dopamine receptor >gi 7...	<u>127</u>	<u>8e-28</u>
	<u>gi 10719976 sp 073810 D2DR_MELGA</u>	D(2) dopamine receptor >gi...	<u>126</u>	<u>1e-27</u>
	<u>gi 17550186 ref NP_508786.1 </u>	(NM_076385) C05E11.7.p [Caenor...	<u>126</u>	<u>2e-27</u>
	<u>gi 16445402 ref NP_387512.1 </u>	(NM_033663) dopamine receptor ...	<u>124</u>	<u>8e-27</u>
	<u>gi 16445398 ref NP_387508.1 </u>	(NM_033659) dopamine receptor ...	<u>123</u>	<u>1e-26</u>

The most closely related human homolog is a dopamine receptor, D2 longer (ACCESSION AAF61479). When this human amino acid sequence was blasted against the Wormpep database, it identified the original T14E8.3 as the second most closely related protein.

10

Sequences producing significant alignments: (bits) Value

15	<u>gi 17562444 ref NP_505478.1 </u>	(NM_073077) dopamine receptor ...	<u>101</u>	<u>7e-22</u>	
	<u>gi 17569809 ref NP_509106.1 </u>	(NM_076705) G-protein coupled ...	<u>99</u>	<u>5e-21</u>	this is
	<u>T14E8.3</u>				
	<u>gi 17567023 ref NP_508760.1 </u>	(NM_076359) G-protein coupled ...	<u>93</u>	<u>3e-19</u>	
	<u>gi 17569449 ref NP_510535.1 </u>	(NM_078134) octopamine recepto...	<u>91</u>	<u>1e-18</u>	
	<u>gi 7504744 pir  T29877</u>	hypothetical protein F59C12.2 - Caen...	<u>87</u>	<u>2e-17</u>	
20	<u>gi 7511648 pir  T37239</u>	serotonin receptor 5-HT2, short spli...	<u>87</u>	<u>3e-17</u>	
	<u>gi 17569447 ref NP_510684.1 </u>	(NM_078283) G-protein coupled ...	<u>86</u>	<u>4e-17</u>	
	<u>gi 17555606 ref NP_497452.1 </u>	(NM_065051) Y22D7AR.13.p [Caen...	<u>86</u>	<u>5e-17</u>	
	<u>gi 17568989 ref NP_508839.1 </u>	(NM_076438) G-protein coupled ...	<u>84</u>	<u>2e-16</u>	
	<u>gi 20198773 gb AAM15552.1 U64603_2 </u>	(U64603) Hypothetical pr...	<u>80</u>	<u>2e-15</u>	
25	<u>gi 17550264 ref NP_508474.1 </u>	(NM_076073) G-protein coupled ...	<u>80</u>	<u>2e-15</u>	
	<u>gi 3025118 sp Q19084 YDBM_CAEEL</u>	PROBABLE G PROTEIN-COUPLED ...	<u>80</u>	<u>3e-15</u>	
	<u>gi 17551400 ref NP_509184.1 </u>	(NM_076783) G-protein coupled ...	<u>80</u>	<u>3e-15</u>	
	<u>gi 17551692 ref NP_508947.1 </u>	(NM_076546) G-protein coupled ...	<u>80</u>	<u>3e-15</u>	

Without being bound to a particular theory, it is possible that this *C. elegans* receptor responds to a neurotransmitter that is regulated by or regulates metabolism and or fat levels. For example, this pathway may correspond to the nematode's autonomic nervous system. Interestingly, the Dopamine D2 receptor in humans has been implicated in obesity (Comings et al., *Biochem Med Metab Biol* 50:176-85, 1993; Contreras et al., *J Hum Hypertens*, 16 Suppl 1, S13-7, 2002) where it functions in the regulation of weight and height. Mutations in this gene may indicate an increased propensity to develop late-onset non-insulin-dependent diabetes mellitus.

### ***C. elegans* Provides A Useful System for Cholesterol-Lowering Drug Screens**

Hydroxymethylglutaryl-CoA (HMG-CoA) reductase is a regulatory enzyme in cholesterol biosynthesis. Inhibitors of HMG-CoA reductase, such as lovastatin or mevinolin, are commonly used to lower human cholesterol. Lovastatin was tested for its effect on *C. elegans* fat accumulation. High fat *lpo-1* mutant nematodes were grown on plates containing 200 µg/ml

mevinolin, an HMG-CoA reductase inhibitor. Fat accumulation was then assessed using Nile Red staining, as previously described. As in humans, lovastatin decreased fat accumulation in *C. elegans*, indicating that *C. elegans* provides a facile, inexpensive, and efficient system in which to identify cholesterol lowering compounds. This experiment demonstrated that natural product 5 libraries can be screened for fat modulators using the Nile Red protocol. Based on our RNAi studies, such fat modulators might target the active site of kinases and phosphatases, the dopamine D2 receptor, fatty acid elongases, and novel proteins.

### Microarrays

10 The global analysis of gene expression using gene chips can provide insights into gene expression perturbations in tissues associated with obesity. Such studies can compare the expression profiles of mammalian fat metabolic regulator genes (e.g., those listed in Table IX, X, XI, XII, XIII, or XIV) in tissues, such as the hypothalamus or fat, of obese and wild-type mice. Genes identified using this method are expected to be fat-level-responsive genes. In fact, 15 transcription factors constitute one class of genes identified via systematic Nile Red RNAi analysis in *C. elegans*. Those genes whose expression is modulated in the obese mice, relative to wild-type control mice, represent important therapeutic targets for further analysis. Such targets could be explored in cell culture models of fat deposition, as well as in obese or lipodystrophic variant humans.

20 Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan et al., U.S. Pat. No. 5,474,796; Schena et al., *Proc. Natl. Acad. Sci.* 93:10614, 1996; Baldeschweiler et al., PCT application WO95/251116, 1995; Shalon, D. et al., PCT application WO95/35505, 1995; Heller et al., *Proc. Natl. Acad. Sci.* 94:2150, 1997; and Heller et al., U.S. Pat. No. 5,605,662; MacBeath et al., *Science* 289:1760-1763, 2000; De Wildt et al., 25 *Nature Biotechnol.* 18, 989-994, 2000; Fung et al., *Curr. Opin. Biotechnol.* 12:65-69, 2001).

### siRNA

Short twenty-one to twenty-five nucleotide double stranded RNAs are effective at down-regulating gene expression in mammalian tissue culture cell lines (Elbashir et al., *Nature* 30 411:494-498, 2001 hereby incorporated by reference). Using such methods, the inactivation of mammalian orthologs (e.g., those listed in Table IX, X, XI, XII, XIII, or XIV) may be analyzed

for fat phenotype. The nucleic acid sequence of mammalian fat metabolism regulator gene orthologs can be used to design small interfering RNAs (siRNAs) that will inactivate mammalian fat metabolism regulator genes for the treatment of obesity or obesity-related disease.

Given the sequence of a mammalian fat metabolism regulator gene, siRNAs may be  
5 designed to inactivate that gene. For example, for a gene that consists of 2000 nucleotides, 1,978 different twenty-two nucleotide oligomers could be designed; this assumes that each oligomer has a two base pair 3' overhang, and that each siRNA is one nucleotide residue from the neighboring siRNA. For RNAi, only a few of these twenty-two nucleotide oligomers would be needed; approximately one dozen siRNAs, evenly spaced across the 2,000 nucleotide gene,  
10 could be sufficient to significantly reduce mammalian gene activity. These siRNAs could be transferred into mammalian cells in culture, and the effect of the siRNAs on the cultured cells fat content would then be assayed using Nile Red, such methods are standard in the art and are described by Elbashir et al., (*Nature* 411:494-498, 2001, hereby incorporated by reference). Alternatively, siRNAs could be injected into an animal, for example, into the blood stream  
15 (McCaffrey et al., *Nature* 418:38-92002). Thus, based on the mammalian genes identified (e.g., those that encode the polypeptides listed in Table IX, X, XI, XII, XIII, or XIV), oligonucleotides may be designed to inhibit mammalian gene activity.

Those siRNAs that are effective in reducing the fat content of cultured cells can be used as therapeutics. The injection of siRNAs corresponding to the DNA sequences of novel  
20 mammalian fat metabolism regulator genes listed in XI would be expected to inactivate those genes, thereby reducing fat levels without adverse side-effects.

### **Druggable Targets**

Enzymes with small molecule substrates have been traditional targets for drug  
25 development. Examples include kinases, phosphatases, lipases, synthases, ABC transporters, nuclear hormone receptors, small molecule receptors, and small molecule transporters. Many small molecule drugs have already been developed. The chemical backbone of drugs designed against a class of enzymes with small substrate molecules, e.g. kinases or nuclear hormone receptors, may be used as a starting point for developing and designing drug targets against other  
30 members within that class of enzymes. The genomic survey described herein has identified a number of enzymes with small molecule substrates that function in regulation of body fat

content. The mammalian orthologs of these worm genes represent targets, in this case for regulation of body fat, that would traditionally be selected for development of small molecule drugs. The mammalian orthologs of the genes listed below appear in Tables XII, XIII, and XIV and are novel candidates for the development of drugs for the treatment of obesity and obesity-related diseases.

Inactivation of the mammalian orthologs of worm fat regulatory genes, for example, by pharmaceuticals that target proteins with small molecule binding sites or small active sites, such as phosphatases, or fatty acid metabolism enzymes, could lead to lower fat levels. For those fat regulatory genes that encode such enzymes, small molecule libraries could be used to screen for the increase or decrease of *in vitro* enzymatic activity of the isolated and purified proteins.

In one working example, a candidate compound that inactivates a kinase could be identified using standard methods to monitor the kinase's biological activity, for example, substrate phosphorylation. A decrease in substrate phosphorylation in the presence of the candidate compound, as compared to substrate phosphorylation in the absence of the candidate compound, identifies that candidate compound as useful in the methods of the invention. In fact, it is reasonable to expect the substrate of that kinase to be present in the lists of fat regulatory genes provided herein, for example, in Tables XII, XIII, and XIV.

In another working example, the biochemical activity of a fatty acid elongase could be monitored *in vitro* using routine methods. A decrease in the biochemical activity of the elongase in the presence of a candidate compound, as compared to the activity of the elongase in the absence of the candidate compound, identifies the candidate compound as useful in the methods of the invention.

In addition to screening synthetic compound libraries, natural product libraries, for example, from fungi, could be screened with isolated and purified proteins expressed *in vitro*. Lovastatin is one example of a natural product that demonstrates that such an approach is advantageous. It is reasonable to expect that fungi and bacteria might produce fat metabolism modulating compounds because they compete with animals for nutrients.

Desirable drug targets are those that have small active sites. These are the most advantageous for drug development, that is, represent druggable targets. Exemplary druggable

targets are shown in Tables XVIII, IX, and XX. This list should not be construed as limiting; other exemplary druggable targets are shown in Tables XII, XIII, and XIV.

**5 Table XVIII RNAi Clones that Reduce Fat Content without Reducing Viability/Growth and have small molecule substrates and their mammalian orthologs are novel targets for drug development**

<b>C. elegans Gene</b>	<b>Brief Description</b>
AH10.1	medium-chain acyl-CoA synthetase
B0285.8	choline/ethanolamine kinase
C01C10.3	phospholipid and glycerol acyltransferase
C06E7.3	S-adenosylmethionine synthetase
C17C3.1	long-chain acyl-coA thioesterase
C24A11.9	trans-prenyltransferase
C31H2.3	4-hydroxyphenylpyruvate dioxygenase
C46H11.2	flavin binding monooxygenase
E01A2.7	glutamate-cysteine ligas/arylesterase
F11E6.5	polyunsaturated fatty acid elongase
F13D11.1	lysosomal acid phosphatase precursor
F23H11.9	CDP-alcohol phosphatidyltransferase
F28H6.3	1-aminocyclopropane-1-carboxylic acid synthase
F43H9.2	serine palmitoyltransferase II
F52B11.2	phosphomannomutase 2
K03B8.3	neutral zinc metallopeptidases
K07C6.4	cytochrome P450 2C2 (P450 PBC2)
K07C6.5	cytochrome P450 2C2 (P450 PBC2)
K09D9.2	cytochrome P450
M28.6	serine beta lactamase-like protein
T04A8.16	calpain-type cystein-protease
T09B4.8	alanine-glyoxylate aminotransferase 2
T12A2.1	chlorohydrolase/histidine degradation
W01C9.4	mitochondrial Δ2,Δ4-dienoyl-CoA reductase
Y49A3A.1	choline/ethanolaminephosphotransferas
Y6B3B.10	lag1 (ceramide synthesis)
C37H5.3	esterase/lipase
F13D12.6	esterase/lipase/Serine carboxypeptidase (S10)
B0280.3	nhr-10(ribose 5-phosphate ketol-isomerase)
C46E10.9	zinc finger, C2H2 type
C47C12.3	zinc finger, C2H2 type/mouse OPR
K08A2.b	hepatocyte nuclear factor 4 receptor type
T09F3.1	zinc finger, C2H2 type
T23F11.4	zinc finger, C2H2 type
W02C12.3	microphthalmia transcription factor
Y116A8C.32	Zn-finger CCHC type transcription factor/ ZFM1
ZK686.4	zinc finger, C2H2 type
B0218.5	serine/threonine kinase

C02F4.2	serine/threonine protein phosphatase (PP2b)
C03D6.3	dual specificity protein phosphatase
C06A1.3	serine/threonine specific protein phosphatase
C16A11.3	serine/threonine protein kinase
C44F1.5	guanylate cyclase
C47D12.1	phosphatidylinositol 3- and 4-kinase/EF-hand family
F41D9.1	RabGAP/TBC domain/SH3 domain
F45H7.4	pim1 serine/threonine-protein kinase
F46G11.3	protein kinase
K10D3.5	protein kinase/adaptor protein
M01B12.5	tyrosine kinase catalytic domain
R107.4	IKK-related kinase epsilon
T05C12.1	serine/threonine protein kinase
T19D2.2	dual specificity protein phosphatase family
Y53C12A.1	serine/threonine protein kinase/membrane associated
ZC302.1	serine/threonine specific protein phosphatase
ZC504.4	Tyrosine kinase and serine/threonine protein kinase
ZK909.3	guanosine-3',5'-bis(diphosphate)-pyrophosphohydrolase
ZK930.1	serine/threonine protein kinase/PI-3
C38C10.1	neurokinin-3 receptor
E02C12.3	Rhodopsin-like GPCR superfamily
C32C4.1	voltage-dependent potassium channel
C13D9.7	sodium/calcium exchanger protein
C34G6.4	ABC transporter
C37A5.1	homology Best's ion exhaenger
C46F11.1	unc-93 protein/ABC-2 type transporter
F15H10.4	lysosomal amino acid transporter
F23F1.6	high affinity cationic amino acid permease
F59F5.1	monocarboxylate transporter/XPCT
K04E7.2	PepT1 oligopeptide symporters
K05F1.6	organic solute carrier family 2/ (OCT1)
ZK682.2	sugar transporter
H27A22.1	glutaminyl cyclase
C15H9.7	kynureninase
C33A12.1	NADH-ubiquinone oxidoreductase B subunit
F14D12.2	cytochrome c family heme-binding site
F20D1.9	mitochondrial carrier proteins
F40H3.5	heparan sulfate sulfotransferase
F11A5.3	similarity to RAB2
W03C9.3	RAB7
F21D5.5	polynucleotide kinase 3' phosphatase

**Table XIX RNAi Clones that Reduce Fat Content and Reduce Viability/Growth and have small molecule substrates and their mammalian orthologs are novel targets for drug development**

GENE NAME	BRIEF DESCRIPTION
Y37D8A.14	Cytochrome c oxidase subunit Va
Y57G11C.12	NADH-ubiquinone oxidoreductase
F28B3.1	Cysteine proteases inhibitor
C23H3.4	serine palmitoyltransferase
E04A4.7	Cytochrome c family heme-binding
F01G10.1	Transketolase
F46E10.1	AMP-dependent synthetase and ligase
F57B9.2	Proline-rich region• Glycosyl hydrolases family 5
H14A12.2	Fumarate lyase
K02F2.2	S-adenosyl-L-homocysteine hydrolase
K06A4.5	3-hydroxyanthranilate 3,4-dioxygenase
T05H4.4	Oxidoreductase/cytochrome B5 reductase
T05H4.5	Oxidoreductase/cytochrome B5 reductase
B0285.1	Eukaryotic protein kinase
C16C2.3	inositol-1,4,5-triphosphate 5-phosphatase
F25H8.3	Neutral zinc metallopeptidase
Y17G7A.2	Zinc finger, C2H2 type
W06D12.2	potassium channel, subfamily K
Y61A9LA_75.a	ABC transporters family
T23F2.1	Glycosyl transferases group 1
F41H10.7	fatty acid elongase (CIG30/Fen1)

5

**Table XX RNAi Clones that Increase Fat Content and have small molecule substrates and their mammalian orthologs are novel targets for drug development**

GENE NAME	BRIEF DESCRIPTION
C33A12.6	UDP-glucuronosyl and UDP-glucosyl transferase
VF13D12L.1	myo-inositol-1-phosphate synthase
C37F5.1	elk-1
C56C10.10	aryl hydrocarbon receptor (Leber congenital amaurosis)
F16B4.9	C4-type steroid receptor zinc finger
K10C3.6	hepatocyte nuclear factor 4 receptor
R11H6.5	interleukin enhancer binding factor 2
C04G2.2	serine/threonine protein kinase/tau tubulin kinase
C24F3.2	glucokinase-associated dual specificity phosphatase
F39B1.1	phosphoinositide 3-kinase
F46C5.6	protein phosphatase PP2A subunit A
F56H11.6	casein kinase/tau-tubulin kinase
R10D12.10	casein kinase/tau-tubulin kinase
T04B2.2	fms/fps protein kinase
T04C9.1	oligophrenin-1 (focal adhesion GTPase)
W03A5.4	guanylate kinase associated protein
ZC513.1	permeability increasing/phospholipid transfer protein

C43H6.9	glutamate receptor
ZC410.4	potassium channel
C18H9.5	sugar transporter
F14E5.1	glucose transporter-3
F52H2.2	amino acid permease

### Transgenic Rodents

Yet another method for assessing the utility of targets, is the use of transgenic rodents that are widely used as mammalian models of obesity. Examples include the following

5 trangenic/mutant mice: *ob/ob*, *db/db*, *fat/fat*, *tubby/tubby*, *-5HTRc/5HTRc*, *MC3R/MC3R*, *MC4R/MC4R*, *BCR3/BCR3*, *11-β-HSD-1/11-β-HSD-1*, *CYP19/CYP19*, *ADR3b/ADR3b*, *Ppara-α/Ppara-α*, *Esr-α/Esr-α*, *Pomc/Pomc*, *Fshr/Fshr*, and agouti mice (Brockmann et al., *Trends in Genetics* 18: 367-376, 2002 and Butler et al., *Trends in Genetics* 17(10):S50-S54, 2001). These mice display hyperphagia and in some cases increased fat deposits. Mammalian fat metabolism 10 regulator genes (e.g., those mammalian genes that encode the polypeptides listed in Table IX, X, XI, XII, XIII, or XIV) can be studied by assaying the fat phenotype of the obese mutant mice having a second mutation in a fat metabolism regulator gene, such as those identified herein (e.g., those novel mammalian genes that encode the polypeptides listed in Table IX, X, XI, XII, XIII, or XIV).

15 Alternatively, obese mice, such as: *ob/ob*, *db/db*, *fat/fat*, *tubby/tubby*, *-5HTRc/5HTRc*, *MC3R/MC3R*, *MC4R/MC4R*, *BCR3/BCR3*, *11-β-HSD-1/11-β-HSD-1*, *CYP19/CYP19*, *ADR3b/ADR3b*, *Ppara-α/Ppara-α*, *Esr-α/Esr-α*, *Pomc/Pomc*, *Fshr/Fshr*, and agouti mice, may be injected with an siRNA (for example, a twenty-one-nucleotide siRNA) that downregulates a mammalian gene identified herein (e.g., those novel mammalian genes that encode the 20 polypeptides listed in Table XII, XIII, or XIV).

### Human Genetics

The many genes that regulate *C. elegans* fat storage may correspond to loci that are variant in human obesity, obesity-related diseases, fat metabolism disorders, or lipodystrophy 25 syndromes. Human obesity-related diseases include, but are not limited to, those diseases which are more common in over-weight individuals, for example, atherosclerosis, heart disease and stroke, noninsulin-dependent diabetes mellitus (type 2 diabetes), several types of cancer that

occur in over-weight women, such as cancer of the uterus, gallbladder, cervix, ovary, breast, or colon, several types of cancer that occur in over-weight men, such as cancer of the colon, rectum, or prostate; joint diseases, such as osteoarthritis I, gout, gallbladder disease or gallstones.

Fat metabolism disorders or lipodystrophy syndromes, include, but are not limited to, 5 diseases of cholesterol and lipid homeostasis (e.g., Tangier disease, familial HDL deficiency, progressive familial intrahepatic cholestasis type 2 and type 3, adrenoleukodystrophy, and sitosterolaemia).

The human orthologs of *C. elegans* fat metabolism regulator genes (e.g., those that encode the polypeptides listed in Table IX, X, XI, XII, XIII, or XIV) may be variant in affected 10 individuals. Such genes could be studied by identifying mutations in the identified candidate genes (e.g., those that encode the polypeptides listed in Table IX, X, XI, XII, XIII, or XIV) in a population of variant humans. Such methods of identification are known to the skilled artisan, and are described Jackson et al. (*Nat. Genet.* 16:303-6. 1997, hereby incorporated by reference).

## 15 Isolation of Additional Fat Metabolism Regulator Genes

Based on the nucleotide and amino acid sequences described herein, the isolation and identification of additional coding sequences of genes regulating fat metabolism is made possible using standard strategies and techniques that are well known in the art.

In one example, fat metabolism regulator polypeptides disclosed herein (e.g., those listed 20 in Tables XII, XIII, or XIV) are used to search a database, as described herein.

In another example, any organism that metabolizes fat can serve as the nucleic acid source for the molecular cloning of such a gene, and these sequences are identified as ones encoding a protein exhibiting structures, properties, or activities associated with fat metabolism regulation, such as the LPO-1, LPO-3, or fat metabolism regulator polypeptides disclosed herein 25 (e.g., those listed in Tables XII, XIII, or XIV).

In one particular example of such an isolation technique, any one of the nucleotide sequences described herein, *lpo-1*, *lpo-3*, or a fat metabolism regulator gene disclosed herein (e.g., those that encode the polypeptides listed in Table XII, XIII, or XIV) may be used, together with conventional methods of nucleic acid hybridization screening. Such hybridization 30 techniques and screening procedures are well known to those skilled in the art and are described,

for example, in Benton and Davis (*Science* 196:180, 1977); Grunstein and Hogness (*Proc. Natl. Acad. Sci., USA* 72:3961, 1975); Ausubel et al. (*Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001); Berger and Kimmel (*Guide to Molecular Cloning Techniques*, 1987, Academic Press, New York); and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York. In one particular example, all or part of the *lpo-1*, *lpo-3*, or nucleic acid sequences that encode the polypeptides listed in Table XII, XIII, or XIV may be used as a probe to screen a recombinant DNA library for genes having sequence identity to the *lpo-1*, *lpo-3* genes or those nucleic acid sequences that encode the polypeptides listed in Table XII, XIII, or XIV. Hybridizing sequences are detected by plaque or colony hybridization according to standard methods.

Alternatively, using all or a portion of the amino acid sequences of LPO-1, LPO-3, or those amino acid sequences listed in Table XII, XIII, or XIV, one may readily design gene-, or nucleic acid sequence specific oligonucleotide probes, including degenerate oligonucleotide probes (i.e., a mixture of all possible coding sequences for a given amino acid sequence). These oligonucleotides may be based upon the sequence of either DNA strand and any appropriate portion of the *lpo-1* or *lpo-3* nucleic acids, or nucleic acid sequences that encode the polypeptides listed in Table XII, XIII, or XIV sequences. General methods for designing and preparing such probes are provided, for example, in Ausubel et al. (supra), and Berger and Kimmel, (*Guide to Molecular Cloning Techniques*, 1987, Academic Press, New York). These oligonucleotides are useful for *lpo-1* or *lpo-3* gene isolation or for the isolation of a gene that encodes a polypeptide listed in Table XII, XIII, or XIV, either through their use as probes capable of hybridizing to *lpo-1* or *lpo-3* gene, or a gene those that encodes a polypeptide listed in Table XII, XIII, or XIV; or as complementary sequences or as primers for various amplification techniques, for example, polymerase chain reaction (PCR) cloning strategies. If desired, a combination of different, detectably-labelled oligonucleotide probes may be used for the screening of a recombinant DNA library. Such libraries are prepared according to methods well known in the art, for example, as described in Ausubel et al. (supra), or they may be obtained from commercial sources.

As discussed above, sequence-specific oligonucleotides may also be used as primers in amplification cloning strategies, for example, using PCR. PCR methods are well known in the

art and are described, for example, in *PCR Technology*, Erlich, ed., Stockton Press, London, 1989; *PCR Protocols: A Guide to Methods and Applications*, Innis et al., eds., Academic Press, Inc., New York, 1990; and Ausubel et al. (*supra*). Primers are optionally designed to allow cloning of the amplified product into a suitable vector, for example, by including appropriate 5 restriction sites at the 5' and 3' ends of the amplified fragment (as described herein). If desired, nucleotide sequences may be isolated using the PCR "RACE" technique, or Rapid Amplification of cDNA Ends (see, e.g., Innis et al. (*supra*)). By this method, oligonucleotide primers based on a desired sequence are oriented in the 3' and 5' directions and are used to generate overlapping PCR fragments. These overlapping 3'- and 5'-end RACE products are combined to produce an 10 intact full-length cDNA. This method is described in Innis et al. (*supra*); and Frohman et al., (*Proc. Natl. Acad. Sci. USA* 85:8998, 1988).

Partial sequences, e.g., sequence tags, are also useful as hybridization probes for identifying full-length sequences, as well as for screening databases for identifying previously unidentified related virulence genes.

15 In general, the invention includes any nucleic acid sequence which may be isolated as described herein or which is readily isolated by homology screening or PCR amplification using any of the nucleic acid sequences disclosed herein (e.g., those listed in Table XII, XIII, or XIV).

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding LPO-1, LPO-3, or the genes that 20 encode the polypeptides listed in Table XII, XIII, or XIV, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide 25 sequence of naturally-occurring *lpo-1*, *lpo-3*, or those nucleic acid sequences that encode the polypeptides listed in Table XII, XIII, or XIV, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode LPO-1, LPO-3, or those polypeptides listed in Table XII, XIII, or XIV, or their variants are preferably capable of hybridizing to the 30 nucleotide sequence of the naturally-occurring *lpo-1*, *lpo-3*, or those polypeptides listed in Table

XII, XIII, or XIV under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding LPO-1, LPO-3, or those polypeptides listed in Table XII, XIII, or XIV, or their derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at 5 which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding LPO-1, LPO-3, or those polypeptides listed in Table XII, XIII, or XIV and their derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable 10 properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode LPO-1, LPO-3, or those polypeptides listed in Table XII, XIII, or XIV, or fragments thereof generated entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into 15 any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding any one of LPO-1, LPO-3, or those polypeptides listed in Table XII, XIII, or XIV, or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of 20 hybridizing to the claimed polynucleotide sequences, and, in particular, to those that encode a polypeptide listed in Table XII, XIII, or XIV, and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) *Methods Enzymol.* 152:399; Kimmel, A. R. (1987) *Methods Enzymol.* 152:507) For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than 25 about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence 25 of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30 °C, 30 more preferably of at least about 37 °C, and most preferably of at least about 42 °C. Varying

additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30 °C in 750 mM

5 NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37 °C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 µg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42 °C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily

10 apparent to those skilled in the art.

The washing steps which follow hybridization can also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM

15 NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include temperature of at least about 25 °C, more preferably of at least about 42 °C, and most preferably of at least about 68 °C. In a preferred embodiment, wash steps will occur at 25°C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will

20 occur at 42°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a most preferred embodiment, wash steps will occur at 68°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art.

Methods for DNA sequencing are well known in the art and may be used to practice any

25 of the embodiments of the invention. The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F. M. (1997) *Short Protocols in Molecular Biology*, John Wiley & Sons, New York N.Y., unit 7.7)

## **Polypeptide Expression**

In general, polypeptides of the invention (e.g., LPO-1, LPO-3, or those listed in Table XII, XIII, or XIV) may be produced by transformation of a suitable host cell with all or part of a polypeptide-encoding nucleic acid or fragment thereof in a suitable expression vehicle.

5        Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems may be used to provide the recombinant protein. The precise host cell used is not critical to the invention. A polypeptide of the invention may be produced in a prokaryotic host (e.g., *E. coli*) or in a eukaryotic host (e.g., *Saccharomyces cerevisiae*, insect cells, e.g., Sf21 cells, or mammalian cells, e.g., NIH 3T3, HeLa, or preferably COS cells). Such 10      cells are available from a wide range of sources (e.g., the American Type Culture Collection, Rockland, MD; also, see, e.g., Ausubel et al., *supra*). The method of transformation or transfection and the choice of expression vehicle will depend on the host system selected. Transformation and transfection methods are described, e.g., in Ausubel et al. (*supra*); expression vehicles may be chosen from those provided, e.g., in Cloning Vectors: A Laboratory Manual 15      (P.H. Pouwels et al., 1985, Supp. 1987).

One particular bacterial expression system for polypeptide production is the *E. coli* pET expression system (Novagen, Inc., Madison, WI). According to this expression system, DNA encoding a polypeptide is inserted into a pET vector in an orientation designed to allow expression. Since the gene encoding such a polypeptide is under the control of the T7 regulatory 20      signals, expression of the polypeptide is achieved by inducing the expression of T7 RNA polymerase in the host cell. This is typically achieved using host strains which express T7 RNA polymerase in response to IPTG induction. Once produced, recombinant polypeptide is then isolated according to standard methods known in the art, for example, those described herein.

Another bacterial expression system for polypeptide production is the pGEX expression 25      system (Pharmacia). This system employs a GST gene fusion system which is designed for high-level expression of genes or gene fragments as fusion proteins with rapid purification and recovery of functional gene products. The protein of interest is fused to the carboxyl terminus of the glutathione S-transferase protein from *Schistosoma japonicum* and is readily purified from bacterial lysates by affinity chromatography using Glutathione Sepharose 4B. Fusion proteins 30      can be recovered under mild conditions by elution with glutathione. Cleavage of the glutathione

S-transferase domain from the fusion protein is facilitated by the presence of recognition sites for site-specific proteases upstream of this domain. For example, proteins expressed in pGEX-2T plasmids may be cleaved with thrombin; those expressed in pGEX-3X may be cleaved with factor Xa.

5 Once the recombinant polypeptide of the invention is expressed, it is isolated, e.g., using affinity chromatography. In one example, an antibody (e.g., produced as described herein) raised against a polypeptide of the invention may be attached to a column and used to isolate the recombinant polypeptide. Lysis and fractionation of polypeptide-harboring cells prior to affinity chromatography may be performed by standard methods (see, e.g., Ausubel et al., *supra*).

10 Once isolated, the recombinant protein can, if desired, be further purified, e.g., by high performance liquid chromatography (see, e.g., Fisher, *Laboratory Techniques In Biochemistry And Molecular Biology*, eds., Work and Burdon, Elsevier, 1980).

15 Polypeptides of the invention, particularly short peptide fragments, can also be produced by chemical synthesis (e.g., by the methods described in *Solid Phase Peptide Synthesis*, 2nd ed., 1984 The Pierce Chemical Co., Rockford, IL). Also included in the invention are polypeptides which are modified in ways which do not abolish their biological activity (assayed, for example as described herein). Such changes may include certain mutations, deletions, insertions, or post-translational modifications, or may involve the inclusion of any of the polypeptides of the invention as one component of a larger fusion protein.

20 The invention further includes analogs of any naturally-occurring polypeptide of the invention. Analogs can differ from the naturally-occurring the polypeptide of the invention by amino acid sequence differences, by post-translational modifications, or by both. Analogs of the invention will generally exhibit at least 85%, more preferably 90%, and most preferably 95% or even 99% identity with all or part of a naturally-occurring amino acid sequence of the invention.

25 The length of sequence comparison is at least 15 amino acid residues, preferably at least 25 amino acid residues, and more preferably more than 35 amino acid residues. Again, in an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between  $e^{-3}$  and  $e^{-100}$  indicating a closely related sequence. Modifications include *in vivo* and *in vitro* chemical derivatization of polypeptides, e.g., acetylation, carboxylation, phosphorylation, or glycosylation; such modifications may occur during

polypeptide synthesis or processing or following treatment with isolated modifying enzymes. Analogs can also differ from the naturally-occurring polypeptides of the invention by alterations in primary sequence. These include genetic variants, both natural and induced (for example, resulting from random mutagenesis by irradiation or exposure to ethanemethylsulfate or by site-specific mutagenesis as described in Sambrook, Fritsch and Maniatis, *Molecular Cloning: A Laboratory Manual* (2d ed.), CSH Press, 1989, or Ausubel et al., *supra*). Also included are cyclized peptides, molecules, and analogs which contain residues other than L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g.,  $\beta$  or  $\gamma$  amino acids.

In addition to full-length polypeptides, the invention also includes fragments of any one of the polypeptides of the invention. As used herein, the term "fragment," means at least 5, preferably at least 20 contiguous amino acids, preferably at least 30 contiguous amino acids, more preferably at least 50 contiguous amino acids, and most preferably at least 60 to 80 or more contiguous amino acids. Fragments of the invention can be generated by methods known to those skilled in the art or may result from normal protein processing (e.g., removal of amino acids from the nascent polypeptide that are not required for biological activity or removal of amino acids by alternative mRNA splicing or alternative protein processing events). The aforementioned general techniques of polypeptide expression and purification can also be used to produce and isolate useful peptide fragments or analogs (described herein).

## 20    **Antibodies**

The polypeptides disclosed herein or variants thereof or cells expressing them can be used as an immunogen to produce antibodies immunospecific for such polypeptides. "Antibodies" as used herein include monoclonal and polyclonal antibodies, chimeric, single chain, simianized antibodies and humanized antibodies, as well as Fab fragments, including the products of an Fab immunoglobulin expression library.

To generate antibodies, a coding sequence for a polypeptide of the invention may be expressed as a C-terminal fusion with glutathione S-transferase (GST) (Smith et al., *Gene* 67:31, 1988). The fusion protein is purified on glutathione-Sepharose beads, eluted with glutathione, cleaved with thrombin (at the engineered cleavage site), and purified to the degree necessary for immunization of rabbits. Primary immunizations are carried out with Freund's complete

adjuvant and subsequent immunizations with Freund's incomplete adjuvant. Antibody titres are monitored by Western blot and immunoprecipitation analyses using the thrombin-cleaved protein fragment of the GST fusion protein. Immune sera are affinity purified using CNBr-Sepharose-coupled protein. Antiserum specificity is determined using a panel of unrelated GST proteins.

5 As an alternate or adjunct immunogen to GST fusion proteins, peptides corresponding to relatively unique immunogenic regions of a polypeptide of the invention may be generated and coupled to keyhole limpet hemocyanin (KLH) through an introduced C-terminal lysine. Antiserum to each of these peptides is similarly affinity purified on peptides conjugated to BSA, and specificity tested in ELISA and Western blots using peptide conjugates, and by Western blot  
10 and immunoprecipitation using the polypeptide expressed as a GST fusion protein.

Alternatively, monoclonal antibodies which specifically bind any one of the polypeptides of the invention are prepared according to standard hybridoma technology (see, e.g., Kohler et al., *Nature* 256:495, 1975; Kohler et al., *Eur. J. Immunol.* 6:511, 1976; Kohler et al., *Eur. J. Immunol.* 6:292, 1976; Hammerling et al., In *Monoclonal Antibodies and T Cell Hybridomas*,  
15 Elsevier, NY, 1981; Ausubel et al., supra). Once produced, monoclonal antibodies are also tested for specific recognition by Western blot or immunoprecipitation analysis (by the methods described in Ausubel et al., supra). Antibodies which specifically recognize the polypeptide of the invention are considered to be useful in the invention; such antibodies may be used, e.g., in an immunoassay. Alternatively monoclonal antibodies may be prepared using the polypeptide of  
20 the invention described above and a phage display library (Vaughan et al., *Nature Biotech* 14:309, 1996).

Preferably, antibodies of the invention are produced using fragments of the polypeptides disclosed herein which lie outside generally conserved regions and appear likely to be antigenic, by criteria such as high frequency of charged residues. In one specific example, such fragments  
25 are generated by standard techniques of PCR and cloned into the pGEX expression vector (Ausubel et al., supra). Fusion proteins are expressed in E. coli and purified using a glutathione agarose affinity matrix as described in Ausubel et al. (supra). To attempt to minimize the potential problems of low affinity or specificity of antisera, two or three such fusions are generated for each protein, and each fusion is injected into at least two rabbits. Antisera are raised by injections in a series, preferably including at least three booster injections.  
30

## Diagnostics

In another embodiment, antibodies which specifically bind any of the polypeptides described herein may be used for the diagnosis of obesity, an obesity-related disease, or a fat metabolism disorder. A variety of protocols for measuring such polypeptides, including 5 immunological methods (such as ELISAs and RIAs) and FACS, are known in the art and provide a basis for diagnosing obesity, an obesity-related disease, or a fat metabolism disorder.

In another aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding *lpo-1*, *lpo-3*, or those encoding a polypeptide listed in Table IX, X, XI, XII, XIII, or XIV, or closely related molecules 10 may be used to identify nucleic acid sequences which encode its gene product. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low), will determine whether the probe identifies only naturally occurring sequences encoding LPO-1, LPO-3, or a polypeptide listed in Table IX, 15 X, XI, XII, XIII, or XIV allelic variants, or related sequences. Hybridization techniques may be used to identify mutations in fat metabolism regulator genes or may be used to monitor expression levels of these genes (for example, by Northern analysis, (Ausubel et al., *supra*).

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as targets in a microarray. The 20 microarray can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents. Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan et al., U.S. Pat. No. 25 5,474,796; Schena et al., *Proc. Natl. Acad. Sci.* 93:10614, 1996; Baldeschweiler et al., PCT application WO95/251116, 1995; Shalon, D. et al., PCT application WO95/35505, 1995; Heller et al., *Proc. Natl. Acad. Sci.* 94:2150, 1997; and Heller et al., U.S. Pat. No. 5,605,662.)

In yet another approach, mammals may be diagnosed for a propensity to a fat metabolism disease or disorder by direct analysis of the sequence of a fat metabolism regulator gene (for

example, by sequence or mismatch detection assays). Exemplary candidates for use as reference wild-type sequences are listed in Table IX, X, XI, XII, XIII, or XIV.

### Screening Assays

- 5 As discussed above, the identified fat metabolism regulator genes, *lpo-1*, *lpo-3*, or those that encode a polypeptide listed in Tables V, VI, VII, IX, X, XI, XII, XIII, and IV modulate the regulation of body fat. Based on this discovery, screening assays were developed to identify compounds that enhance or inhibit the action of a polypeptide or the expression of a nucleic acid sequence of the invention. The method of screening may involve high-throughput techniques.
- 10 In addition, these screening techniques may be carried out in cultured cells or in animals (such as nematodes).

Any number of methods are available for carrying out such screening assays. In one working example, candidate compounds are added at varying concentrations to the culture medium of cultured cells expressing one of the nucleic acid sequences of the invention. Gene expression is then measured, for example, by standard Northern blot analysis (Ausubel et al., supra) or RT-PCR, using any appropriate fragment prepared from the nucleic acid as a hybridization probe. The level of gene expression in the presence of the candidate compound is compared to the level measured in a control culture medium lacking the candidate molecule. A compound which promotes an increase in the expression of *lpo-1*, *lpo-3*, or a nucleic acid that encodes a polypeptide listed in Tables V, VI, VII, IX, X, XI, XII, XIII, and IV or functional equivalent is considered useful in the invention; such a molecule may be used, for example, as a therapeutic to delay or ameliorate human diseases associated with obesity, an obesity-related disease, or a fat metabolism disorder. Such cultured cells include nematode cells (for example, *C. elegans* cells), mammalian, or insect cells.

25 In another working example, the effect of candidate compounds may be measured at the level of polypeptide production using the same general approach and standard immunological techniques, such as Western blotting or immunoprecipitation with an antibody specific for a fat metabolism regulator polypeptide, such as LPO-1, LPO-3, or a polypeptide listed in Table IX, X, XI, XII, XIII, or XIV. For example, immunoassays may be used to detect or monitor the expression of at least one of the polypeptides of the invention in an organism. Polyclonal or

monoclonal antibodies (produced as described above) which are capable of binding to such a polypeptide may be used in any standard immunoassay format (e.g., ELISA, Western blot, or RIA assay) to measure the level of the polypeptide. A compound which promotes an increase in the expression of the polypeptide is considered particularly useful. Again, such a molecule may  
5 be used, for example, as a therapeutic to delay or ameliorate human diseases associated with excess body weight or obesity as is described above.

In yet another working example, candidate compounds may be screened for those which specifically bind to and agonize or antagonize LPO-1, LPO-3, or a polypeptide listed in Table V, VI, VII, IX, X, XI, XII, XIII, or XIV. The efficacy of such a candidate compound is dependent  
10 upon its ability to interact with LPO-1, LPO-3, or a polypeptide listed in Table V, VI, VII, IX, X, XI, XII, XIII, or XIV or a functional equivalent thereof. Such an interaction can be readily assayed using any number of standard binding techniques and functional assays (e.g., those described in Ausubel et al., *supra*). For example, a candidate compound may be tested *in vitro*  
15 for interaction and binding with a polypeptide of the invention and its ability to modulate body fat metabolism may be assayed by any standard assay (e.g., those described herein).

In one particular working example, a candidate compound that binds to a polypeptide (e.g., LPO-1, LPO-3, or a polypeptide listed in Table V, VI, VII, IX, X, XI, XII, XIII, or XIV) may be identified using a chromatography-based technique. For example, a recombinant polypeptide of the invention may be purified by standard techniques from cells engineered to  
20 express the polypeptide (e.g., those described above) and may be immobilized on a column. A solution of candidate compounds is then passed through the column, and a compound specific for the fat metabolism regulator polypeptide is identified on the basis of its ability to bind to the fat metabolism regulator polypeptide and be immobilized on the column. To isolate the compound, the column is washed to remove non-specifically bound molecules, and the  
25 compound of interest is then released from the column and collected. Compounds isolated by this method (or any other appropriate method) may, if desired, be further purified (e.g., by high performance liquid chromatography). In addition, these candidate compounds may be tested for their ability to affect fat metabolism (e.g., as described herein). Compounds isolated by this approach may also be used, for example, as therapeutics to delay or ameliorate human diseases  
30 associated with excess body weight or obesity. Compounds which are identified as binding to fat

metabolism regulator polypeptides with an affinity constant less than or equal to 10 mM are considered particularly useful in the invention.

Potential agonists and antagonists include organic molecules, peptides, peptide mimetics, polypeptides, nucleic acids, and antibodies that bind to a nucleic acid sequence or polypeptide of

5 the invention (e.g., fat metabolism regulator polypeptides) and thereby increase its activity.

Potential agonists also include small molecules that bind to and occupy the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented.

Each of the DNA sequences provided herein may also be used in the discovery and

10 development of fat metabolism regulator compounds. The encoded protein, upon expression, can be used as a target for the screening of fat metabolism regulating drugs. Additionally, the DNA sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other translation facilitating sequences of the respective mRNA can be used to construct antisense sequences to control the expression of the coding sequence of interest. Such sequences

15 may be isolated by standard techniques (Ausubel et al., *supra*).

The antagonists and agonists of the invention may be employed, for instance, to delay or ameliorate human diseases associated with obesity, an obesity-related disease, or a fat metabolism disorder.

Optionally, compounds identified in any of the above-described assays may be confirmed

20 as useful in delaying or ameliorating human diseases associated with excess body weight or obesity, an obesity-related disease, or a fat metabolism disorder in either standard tissue culture methods (e.g. Nile Red staining of fat storage in cultured cells) or animal models (e.g., naturally occurring rodent mutants, such as, for example, Ob (leptin), db (leptin receptor), fat-1 (carboxypeptidase E), 5-HT<sub>2</sub> (serotonin receptor) and tubby and, if successful, may be used as

25 therapeutics for the treatment of obesity or disorders related to fat metabolism.

Small molecules of the invention preferably have a molecular weight below 2,000 daltons, more preferably between 300 and 1,000 daltons, and most preferably between 400 and 700 daltons. It is preferred that these small molecules are organic molecules.

## Test Compounds and Extracts

In general, compounds capable of delaying or ameliorating human diseases associated with obesity, an obesity-related disease, or a fat metabolism disorder are identified from large libraries of both natural product or synthetic (or semi-synthetic) extracts or chemical libraries

5 according to methods known in the art. Those skilled in the field of drug discovery and development will understand that the precise source of test extracts or compounds is not critical to the screening procedure(s) of the invention. Compounds used in screens may include known compounds (for example, known therapeutics used for other diseases or disorders).

Alternatively, virtually any number of unknown chemical extracts or compounds can be screened  
10 using the methods described herein. Examples of such extracts or compounds include, but are not limited to, plant-, fungal-, prokaryotic- or animal-based extracts, fermentation broths, and synthetic compounds, as well as modification of existing compounds. Numerous methods are also available for generating random or directed synthesis (e.g., semi-synthesis or total synthesis)  
15 of any number of chemical compounds, including, but not limited to, saccharide-, lipid-, peptide-, and nucleic acid-based compounds. Synthetic compound libraries are commercially available from Brandon Associates (Merrimack, NH) and Aldrich Chemical (Milwaukee, WI).

Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant, and animal extracts are commercially available from a number of sources, including Biotics (Sussex, UK), Xenova (Slough, UK), Harbor Branch Oceanographics Institute (Ft. Pierce, FL), and PharmaMar,  
20 U.S.A. (Cambridge, MA). In addition, natural and synthetically produced libraries are produced, if desired, according to methods known in the art, e.g., by standard extraction and fractionation methods. Furthermore, if desired, any library or compound is readily modified using standard chemical, physical, or biochemical methods.

In addition, those skilled in the art of drug discovery and development readily understand  
25 that methods for dereplication (e.g., taxonomic dereplication, biological dereplication, and chemical dereplication, or any combination thereof) or the elimination of replicates or repeats of materials already known for their fat metabolism regulating activity should be employed whenever possible.

When a crude extract is found to have a fat metabolism regulating activity, or a binding  
30 activity, further fractionation of the positive lead extract is necessary to isolate chemical

constituents responsible for the observed effect. Thus, the goal of the extraction, fractionation, and purification process is the careful characterization and identification of a chemical entity within the crude extract having fat metabolism regulating activity. Methods of fractionation and purification of such heterogenous extracts are known in the art. If desired, compounds shown to  
5 be useful agents to delay or ameliorate human diseases associated with obesity, an obesity-related disease, or a fat metabolism disorder are chemically modified according to methods known in the art.

### **RNA interference**

RNAi is a form of post-transcriptional gene silencing initiated by the introduction of double-stranded RNA (dsRNA). Elbashir et al. reported that twenty-one-nucleotide RNA duplexes introduced into cultured mammalian cells could elicit gene-specific silencing (*Nature* 411:494-498, 2001). Based on these results, one would predict that a double stranded RNA corresponding to one of the fat metabolism regulator genes described herein (e.g., those that encode a polypeptide listed in Table XII, XIII, or XIV) could be used to specifically silence fat metabolism regulator gene expression. To this end, the nucleic acids described herein are contemplated to be employed as double-stranded RNA molecules.

10

### **Pharmaceutical Therapeutics**

The invention provides a simple means for identifying compounds (including peptides, small molecule inhibitors, and mimetics) capable of delaying or ameliorating human diseases associated with obesity, an obesity-related disease, or a fat metabolism disorder. Accordingly, a  
15 chemical entity discovered to have medicinal value using the methods described herein is useful as a drug or as information for structural modification of existing fat metabolism regulating compounds, e.g., by rational drug design. Such methods are useful for screening compounds having an effect on a variety of conditions involving the dysregulation of body weight, fat metabolism, energy metabolism, obesity, including, but not limited to, atherosclerosis, type II diabetes mellitus, osteoarthritis of body joints, diseases of cholesterol and lipid homeostasis (e.g.,  
20 Tangier disease, familial HDL deficiency, progressive familial intrahepatic cholestasis type 2 and type 3, adrenoleukodystrophy, and sitosterolaemia).

For therapeutic uses, the compositions or agents identified using the methods disclosed herein may be administered systemically, for example, formulated in a pharmaceutically-acceptable buffer such as physiological saline. Treatment may be accomplished directly, e.g., by treating the animal with antagonists which disrupt, suppress, attenuate, or neutralize the

5 biological events associated with a fat metabolism regulator polypeptide. Preferable routes of administration include, for example, subcutaneous, intravenous, interperitoneally, intramuscular, or intradermal injections that provide continuous, sustained levels of the drug in the patient.

Treatment of human patients or other animals will be carried out using a therapeutically effective amount of a fat metabolism regulator agent in a physiologically-acceptable carrier. Suitable

10 carriers and their formulation are described, for example, in Remington's Pharmaceutical Sciences by E.W. Martin. The amount of the fat metabolism regulator agent to be administered varies depending upon the manner of administration, the age and body weight of the patient, and with the type of disease and extensiveness of the disease. Generally, amounts will be in the range of those used for other agents used in the treatment of other diseases associated with fat

15 metabolism or obesity, although in certain instances lower amounts will be needed because of the increased specificity of the compound. A compound is administered at a dosage that inhibits obesity, an obesity-related disease, or a fat metabolism disorder

### **Use of Transgenic and Knockout Animals in Diagnosis or Drug Screening**

20 The present invention also includes transgenic and knock-out animals that may be used as research tools to determine genetic and physiological features of obesity, an obesity-related disease, or a fat metabolism disorder, and for identifying compounds that can affect such diseases or disorders. Such animals may include, for example, transgenic or knockout nematodes or rodents. Knockout animals include, for example, mutant animals that are either

25 homozygous or heterozygous, for a deletion in a fat metabolism regulator gene (e.g., a gene listed in Tables V-XIV). Knockout animals also include animals where this normal gene has been inactivated or removed and replaced with a known polymorphic or other mutant allele of this gene. These animals can serve as a model system for the risk of acquiring a disease that is associated with a particular allele. In general, the method of identifying a fat metabolism

30 regulator gene involves comparing the presence, absence, or level of expression of genes, either

at the RNA level or at the protein level, in tissue from a transgenic or knock-out animal and in tissue from a matching non-transgenic or knock-out animal. Standard techniques for detecting RNA expression, e.g., by Northern blotting, or protein expression, e.g., by Western blotting, are well known in the art. Differences between animals such as the presence, absence, or level of expression of a gene indicate that the expression of the gene is a marker associated with a disorder. Identification of such markers are useful since they are candidate therapeutic targets. Identification of markers can take several forms.

One method by which molecular markers may be identified is by use of directed screens. Patterns of accumulation of a variety of molecules can be surveyed using immunohistochemical methods. Screens directed at analyzing expression of specific genes or groups of molecules implicated in obesity, an obesity-related disease, or a fat metabolism disorder can be continued during the life of the transgenic or knockout animal. Expression can be monitored by immunohistochemistry as well as by protein and RNA blotting techniques.

Alternatively, molecular markers may be identified using genomic screens. For example, tissue can be recovered from young transgenic or knockout animals and older transgenic or knockout animals, and compared with similar material recovered from age-matched normal littermate controls to catalog genes that are induced or repressed as obesity, an obesity-related disease, or a fat metabolism disorder is initiated, and as obesity, an obesity-related disease, or a fat metabolism disorder progresses to its final stages. These surveys will generally include cellular populations present in the affected tissue.

This analysis can also be extended to include an assessment of the effects of various treatments on differential gene expression (DGE). The information derived from the surveys of DGE can ultimately be correlated with obesity, an obesity-related disease, or a fat metabolism disorder initiation and progression in the transgenic or knockout animals.

To assess the effectiveness of a treatment paradigm, a transgene, such as a mutant of any of the nucleic acid sequences described herein, may be conditionally expressed (e.g., in a tetracycline sensitive manner). For example, the promoter for this gene may contain a sequence that is regulated by tetracycline and expression of the gene product ceases when tetracycline is administered to the mouse. In this example, a tetracycline-binding operator, tetO, is regulated by the addition of tetracycline, or an analog thereof, to the organism's water or diet. The tetO may

be operably-linked to a coding region, for example, a wild-type or mutant nucleic acid sequence described herein. The system also may include a tetracycline transactivator (tTA), which contains a DNA binding domain that is capable of binding the tetO as well as a polypeptide capable of repressing transcription from the tetO (e.g., the tetracycline repressor (tetR)), and may 5 be further coupled to a transcriptional activation domain (e.g., VP16). When the tTA binds to the tetO sequences, in the absence of tetracycline, transcription of the target gene is activated. However, binding of tetracycline to the tTA prevents activation. Thus, a gene operably-linked to a tetO is expressed in the absence of tetracycline and is repressed in its presence. Alternatively, this system could be modified such that a gene is expressed in the presence of tetracycline and 10 repressed in its absence. Tetracycline regulatable systems are well known to those skilled in the art and are described in, for example, WO 94/29442, WO 96/40892, WO 96/01313, and Yamamoto *et al.* (*Cell* 101:57-66, 2000).

In addition, the knockout organism may be a conditional, i.e., somatic, knockout. For 15 example, FRT sequences may be introduced into the organism so that they flank the gene of interest. Transient or continuous expression of the FLP protein may then be used to induce site-directed recombination, resulting in the excision of the gene of interest. The use of the FLP/FRT system is well established in the art and is described in, for example, U.S. Patent Number 5,527,695, and in Lyznik *et al.* (*Nucleic Acid Research* 24:3784-3789, 1996).

Conditional, i.e., somatic knockout organisms may also be produced using the Cre-lox 20 recombination system. Cre is an enzyme that excises DNA between two recognition sites termed loxP. The *cre* transgene may be under the control of an inducible, developmentally regulated, tissue specific, or cell-type specific promoter. In the presence of Cre, the gene, for example a nucleic acid sequence described herein, flanked by loxP sites is excised, generating a knockout. This system is described, for example, in Kilby *et al.* (*Trends in Genetics* 9:413-421, 1993).

25 Particularly desirable is a mouse model wherein an altered nucleic acid sequence described herein is expressed in specific cells of the transgenic mouse such that the transgenic mouse develops obesity, an obesity-related disease, or fat metabolism disorder. In addition, cell lines from these mice may be established by methods standard in the art.

Construction of transgenes can be accomplished using any suitable genetic engineering 30 technique, such as those described in Ausubel *et al.* (*Current Protocols in Molecular Biology*,

John Wiley & Sons, New York, 2000). Many techniques of transgene construction and of expression constructs for transfection or transformation in general are known and may be used for the disclosed constructs.

One skilled in the art will appreciate that a promoter is chosen that directs expression of the chosen gene in the tissue in which a disease or disorder is expected to develop. For example, as noted above, any promoter that regulates expression of a nucleic acid sequence described herein can be used in the expression constructs of the present invention. One skilled in the art would be aware that the modular nature of transcriptional regulatory elements and the absence of position-dependence of the function of some regulatory elements, such as enhancers, make modifications such as, for example, rearrangements, deletions of some elements or extraneous sequences, and insertion of heterologous elements possible. Numerous techniques are available for dissecting the regulatory elements of genes to determine their location and function. Such information can be used to direct modification of the elements, if desired. It is desirable, however, that an intact region of the transcriptional regulatory elements of a gene is used. Once a suitable transgene construct has been made, any suitable technique for introducing this construct into embryonic cells can be used.

Animals suitable for transgenic experiments can be obtained from standard commercial sources such as Taconic (Germantown, N.Y.). Many strains are suitable, but Swiss Webster (Taconic) female mice are desirable for embryo retrieval and transfer. B6D2F (Taconic) males can be used for mating and vasectomized Swiss Webster studs can be used to stimulate pseudopregnancy. Vasectomized mice and rats are publicly available from the above-mentioned suppliers. However, one skilled in the art would also know how to make a transgenic mouse or rat. An example of a protocol that can be used to produce a transgenic animal is provided below.

## 25 Production Of Transgenic Mice And Rats

The following is but one desirable means of producing transgenic mice. This general protocol may be modified by those skilled in the art.

Female mice six weeks of age are induced to superovulate with a 5 IU injection (0.1 cc, IP) of pregnant mare serum gonadotropin (PMSG; Sigma) followed 48 hours later by a 5 IU injection (0.1 cc, IP) of human chorionic gonadotropin (hCG, Sigma). Females are placed

together with males immediately after hCG injection. Twenty-one hours after hCG injection, the mated females are sacrificed by CO<sub>2</sub> asphyxiation or cervical dislocation and embryos are recovered from excised oviducts and placed in Dulbecco's phosphate buffered saline with 0.5% bovine serum albumin (BSA, Sigma). Surrounding cumulus cells are removed with  
5 hyaluronidase (1 mg/ml). Pronuclear embryos are then washed and placed in Earle's balanced salt solution containing 0.5% BSA (EBSS) in a 37.5°C incubator with humidified atmosphere at 5% CO<sub>2</sub>, 95% air until the time of injection. Embryos can be implanted at the two-cell stage.

Randomly cycling adult female mice are paired with vasectomized males. Swiss Webster or other comparable strains can be used for this purpose. Recipient females are mated at the  
10 same time as donor females. At the time of embryo transfer, the recipient females are anesthetized with an intraperitoneal injection of 0.015 ml of 2.5% avertin per gram of body weight. The oviducts are exposed by a single midline dorsal incision. An incision is then made through the body wall directly over the oviduct. The ovarian bursa is then torn with watchmakers forceps. Embryos to be transferred are placed in DPBS (Dulbecco's phosphate  
15 buffered saline) and in the tip of a transfer pipet (about 10 to 12 embryos). The pipet tip is inserted into the infundibulum and the embryos are transferred. After the transferring the embryos, the incision is closed by two sutures.

A desirable procedure for generating transgenic rats is similar to that described above for mice (Hammer *et al.*, *Cell* 63:1099-112, 1990). For example, thirty-day old female rats are given  
20 a subcutaneous injection of 20 IU of PMSG (0.1 cc) and 48 hours later each female placed with a proven, fertile male. At the same time, 40-80 day old females are placed in cages with vasectomized males. These will provide the foster mothers for embryo transfer. The next morning females are checked for vaginal plugs. Females who have mated with vasectomized males are held aside until the time of transfer. Donor females that have mated are sacrificed  
25 (CO<sub>2</sub> asphyxiation) and their oviducts removed, placed in DPBA (Dulbecco's phosphate buffered saline) with 0.5% BSA and the embryos collected. Cumulus cells surrounding the embryos are removed with hyaluronidase (1 mg/ml). The embryos are then washed and placed in EBSs (Earle's balanced salt solution) containing 0.5% BSA in a 37.5°C incubator until the time of microinjection.

Once the embryos are injected, the live embryos are moved to DPBS for transfer into foster mothers. The foster mothers are anesthetized with ketamine (40 mg/kg, IP) and xulazine (5 mg/kg, IP). A dorsal midline incision is made through the skin and the ovary and oviduct are exposed by an incision through the muscle layer directly over the ovary. The ovarian bursa is  
5 torn, the embryos are picked up into the transfer pipet, and the tip of the transfer pipet is inserted into the infundibulum. Approximately 10 to 12 embryos are transferred into each rat oviduct through the infundibulum. The incision is then closed with sutures, and the foster mothers are housed singly.

## 10 Generation Of Knockout Mice

The following is but one example for the generation of a knockout mouse and the protocol may be readily adapted or modified by those skilled in the art.

Embryonic stem cells (ES), for example,  $10^7$  AB1 cells, may be electroporated with 25 µg targeting construct in 0.9 ml PBS using a Bio-Rad Gene Pulser (500 µF, 230 V). The cells  
15 may then be plated on one or two 10-cm plates containing a monolayer of irradiated STO feeder cells. Twenty-four hours later, they may be subjected to G418 selection (350 µg/ml, Gibco) for 9 days. Resistant clones may then be analyzed by Southern blotting after *Hind* III digestion, using a probe specific to the targeting construct. Positive clones are expanded and injected into C57BL/6 blastocysts. Male chimeras may be back-crossed to C57BL/6 females. Heterozygotes  
20 may be identified by Southern blotting and intercrossed to generate homozygotes.

The targeting construct may result in the disruption of the gene of interest, e.g., by insertion of a heterologous sequence containing stop codons, or the construct may be used to replace the wild-type gene with a mutant form of the same gene, e.g., a “knock-in.” Furthermore, the targeting construct may contain a sequence that allows for conditional  
25 expression of the gene of interest. For example, a sequence may be inserted into the gene of interest that results in the protein not being expressed in the presence of tetracycline. Such conditional expression of a gene is described in, for example, Yamamoto *et al.* (*Cell* 101:57-66, 2000)).

All publications and references, including but not limited to patents and patent  
30 applications, cited in this specification are herein incorporated by reference in their entirety as if

each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references.

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What is claimed is: